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(54) Title: INHIBITORS OF PRENYL–PROTEIN TRANSFERASES

(57) Abstract

The present invention comprises piperazine–containing compounds which inhibit prenyl–protein transferases. In particular, the invention relates to prenyl–protein transferase inhibitors which are efficacious in vivo as inhibitors of geranylgeranyl–protein transferase type I (GGTase–I) and that inhibit the cellular processing of both the H–Ras protein and the K4B–Ras protein. Such therapeutic compounds are useful in the treatment of cancer.

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TITLE OF THE INVENTION

INHIBITORS OF PRENYL-PROTEIN TRANSFERASES

BACKGROUND OF THE INVENTION

The present invention relates to certain compounds that are useful for the inhibition of prenyl-protein transferases and the treatment of cancer. In particular, the invention relates to prenyl-protein transferase inhibitors which are efficacious in vivo as inhibitors of geranylgeranyl-protein transferase type I (GGTase-I) and that inhibit the cellular processing of both the H-Ras protein and the K4B-Ras protein.

Prenylation of proteins by prenyl-protein transferases represents a class of post-translational modification (Glomset, J. A., Gelb, M. H., and Farnsworth, C. C. (1990). Trends Biochem. Sci. 15, 139-142; Maltese, W. A. (1990). FASEB J. 4, 3319-3328). This modification typically is required for the membrane localization and function of these proteins. Prenylated proteins share characteristic C-terminal sequences including CAAX (C, Cys; A, an aliphatic amino acid; X, another amino acid), XXCC, or XCXC. Three post-translational processing steps have been described for proteins having a C-terminal CAAX sequence: addition of either a 15 carbon (farnesyl) or 20 carbon (geranylgeranyl) isoprenoid to the Cys residue, proteolytic cleavage of the last 3 amino acids, and methylation of the new C-terminal carboxylate (Cox, A. D. and Der, C. J. (1992a). Critical Rev. Oncogenesis 3:365-400; Newman, C. M. H. and Magee, A. I. (1993). Biochim. Biophys. Acta 1155:79-96). Some proteins may also have a fourth modification: palmitoylation of one or two Cys residues N-terminal to the farnesylated Cys. While some mammalian cell proteins terminating in XCXC are carboxymethylated, it is not clear whether carboxy methylation follows prenylation of proteins terminating with a XXCC motif (Clarke, S. (1992). Annu. Rev. Biochem. 61, 355-386). For all of the prenylated proteins, addition of the isoprenoid is the first step and is required for the subsequent steps (Cox, A. D. and Der, C. J. (1992a). Critical Rev. Oncogenesis 3:365-400; Cox, A. D. and Der, C. J. (1992b) Current Opinion Cell Biol. 4:1008-1016).

Three enzymes have been described that catalyze protein prenylation: farnesyl-protein transferase (FPTase), geranylgeranyl-protein transferase type I (GGPTase-I), and geranylgeranyl-protein transferase type-II (GGPTase-II, also called Rab GGPTase). These enzymes are found in both yeast and mammalian cells (Clarke, 1992; Schafer, W. R. and Rine, J. (1992) Annu. Rev. Genet. 30:209-237).

Each of these enzymes selectively uses farnesyl diphosphate or geranyl-geranyl diphosphate as the isoprenoid donor and selectively recognizes the protein substrate. FPTase farnesylates CaaX-containing proteins that end with Ser, Met, Cys, Gln or Ala. For FPTase, CaaX tetrapeptides comprise the minimum region required for interaction of the protein substrate with the enzyme. The enzymological characterization of these three enzymes has demonstrated that it is possible to selectively inhibit one with little inhibitory effect on the others (Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O'Hara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L., and Gibbs, J. B., J. Biol. Chem., 266:17438 (1991), U.S. Pat. No. 5,470,832).

The prenylation reactions have been shown genetically to be essential for the function of a variety of proteins (Clarke, 1992; Cox and Der, 1992a; Gibbs, J. B. (1991). Cell 65: 1-4; Newman and Magee, 1993; Schafer and Rine, 1992). This requirement often is demonstrated by mutating the CaaX Cys acceptors so that the proteins can no longer be prenylated. The resulting proteins are devoid of their central biological activity. These studies provide a genetic "proof of principle" indicating that inhibitors of prenylation can alter the physiological responses regulated by prenylated proteins.

The Ras protein is part of a signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein. In the inactive state,
Ras is bound to GDP. Upon growth factor receptor activation, Ras is induced to exchange GDP for GTP and undergoes a conformational change. The GTP-bound form of Ras propagates the growth stimulatory signal until the signal is terminated by the intrinsic GTPase activity of Ras, which returns the protein to its inactive GDP bound form (D.R. Lowy and D.M. Willumsen, Ann. Rev. Biochem. 62:851-891 (1993)). Activation of Ras leads to activation of multiple intracellular signal transduction pathways, including the MAP Kinase pathway and the Rho/Rac pathway (Joneson et al., Science 271:810-812).

Mutated ras genes are found in many human cancers, including colorectal carcinoma, exocrine pancreatic carcinoma, and myeloid leukemias. The protein products of these genes are defective in their GTPase activity and constitutively transmit a growth stimulatory signal.

The Ras protein is one of several proteins that are known to undergo post-translational modification. Farnesyl-protein transferase utilizes farnesyl pyrophosphate to covalently modify the Cys thiol group of the Ras CAAX box with a farnesyl group (Reiss et al., Cell, 62:81-88 (1990); Schaber et al., J. Biol. Chem., 265:14701-14704 (1990); Schafer et al., Science, 249:1133-1139 (1990); Manne et al., Proc. Natl. Acad. Sci USA, 87:7541-7545 (1990)).

Ras must be localized to the plasma membrane for both normal and oncogenic functions. At least 3 post-translational modifications are involved with Ras membrane localization, and all 3 modifications occur at the C-terminus of Ras. The Ras C-terminus contains a sequence motif termed a "CAAX" or "Cys-Aaa^1-Aaa^2-Xaa" box (Cys is cysteine, Aaa is an aliphatic amino acid, the Xaa is any amino acid) (Willumsen et al., Nature 310:583-586 (1984)). Depending on the specific sequence, this motif serves as a signal sequence for the enzymes farnesyl-protein transferase or geranylgeranyl-protein transferase, which catalyze the alkylation of the cysteine residue of the CAAX motif with a C_{15} or C_{20} isoprenoid, respectively. (S. Clarke., Ann. Rev. Biochem. 61:355-386 (1992); W.R. Schafer and J. Rine, Ann. Rev. Genetics 30:209-237 (1992)). Direct inhibition of farnesyl-protein transferase would be more specific and attended by fewer side effects than would occur with the required dose of a general inhibitor of isoprene biosynthesis.

Other farnesylated proteins include the Ras-related GTP-binding proteins such as RhoB, fungal mating factors, the nuclear lamins, and the gamma subunit of transducin. James, et al., J. Biol. Chem. 269, 14182 (1994) have identified a peroxisome associated protein Pxf which is also farnesylated. James, et al., have also suggested that there are farnesylated proteins of unknown structure and function in addition to those listed above.

Inhibitors of farnesyl-protein transferase (FPTase) have been described in two general classes. The first class includes analogs of farnesyl diphosphate (FPP), while the second is related to protein substrates (e.g., Ras) for the enzyme. The peptide derived inhibitors that have been described are generally cysteine containing molecules that are related to the CAAX motif that is the signal for protein prenylation. (Schaber et al., ibid; Reiss et. al., ibid; Reiss et al., PNAS, 88:732-736 (1991)). Such inhibitors may inhibit protein prenylation while serving as alternate substrates for the farnesyl-protein transferase enzyme, or may be purely competitive inhibitors (U.S. Patent 5,141,851, University of Texas; N.E. Kohl et al., Science, 260:1934-1937 (1993); Graham, et al., J. Med. Chem., 37, 725 (1994)).

Mammalian cells express four types of Ras proteins (H-, N-, K4A-, and K4B-Ras) among which K4B-Ras is the most frequently mutated form of Ras in human cancers. The genes that encode these proteins are abbreviated H-ras, N-ras , K4A-ras and K4B-ras respectively. H-ras is an abbreviation for Harvey-ras. K4A-ras and K4B-ras are abbreviations for the Kirsten splice variants of ras that contain the 4A and 4B exons, respectively. Inhibition of farnesyl-protein transferase has been shown to block the growth of H-ras-transformed cells in soft agar and to modify other aspects of their transformed phenotype. It has also been demonstrated that certain inhibitors of farnesyl-protein transferase selectively block the processing of the H-Ras oncoprotein intracellularly (N.E. Kohl et al., Science, 260:1934-1937 (1993) and G.L. James et al., Science, 260:1937-1942 (1993)). Recently, it has been shown that an inhibitor of farnesyl-protein transferase blocks the growth of H-ras-dependent tumors in nude mice (N.E. Kohl et al., Proc. Natl. Acad. Sci U.S.A., 91:9141-9145 (1994) and induces regression of mammary and salivary carcinomas in H-ras transgenic mice (N.E. Kohl et al., Nature Medicine, 1:792-797 (1995).

Indirect inhibition of farnesyl-protein transferase in vivo has been demonstrated with lovastatin (Merck & Co., Rahway, NJ) and compactin (Hancock et al., ibid; Casey et al., ibid; Schafer et al., Science 245:379 (1989)). These drugs inhibit HMG-CoA reductase, the rate limiting enzyme for the production of polyisoprenoids includ-ing farnesyl pyrophosphate. Inhibition of farnesyl pyrophosphate biosynthesis by inhibiting HMG-CoA reductase blocks Ras membrane localization in cultured cells.

It has been disclosed that the lysine-rich region and terminal CVIM sequence of the C-terminus of K-RasB confer resistance to inhibition of the cellular processing of that protein by certain selective FPTase inhibitors. (James, et al., J. Biol. Chem. 270, 6221 (1995) Those FPTase inhibitors were effective in inhibiting the processing of H-Ras proteins. James et al., suggested that prenylation of the K4B-Ras protein by GGTase-I contributed to the resistance to the selective FPTase inhibitors.

Selective inhibitors of GGTase-I have been previously disclosed (see for example U.S. Pat. No. 5,470,832, issued November 28, 1995). Other compounds have been described as selective inhibitors of GGTase-I (see for example PCT Publication No. WO 96/21456). Combinations of a selective inhibitor of FPTase and a selective inhibitor of GGTase-I have been disclosed as useful in the treatment of cancer (PCT Publication No. WO 97/34664).

Several groups of scientists have recently disclosed compounds that are non-selective FPTase/GGTase-I inhibitors. (Nagasu et al. Cancer Research, 55:5310-5314 (1995); PCT application WO 95/25086).

It is the object of the instant invention to provide a prenyl-protein transferase inhibitor which is efficacious in vivo as an inhibitor of geranylgeranyl-protein transferase type I (GGTase-I), also known as CAAX GGTase.

It is also the object of the present invention to provide a compound which inhibits the cellular processing of both the H-Ras protein and the K4B-Ras protein.

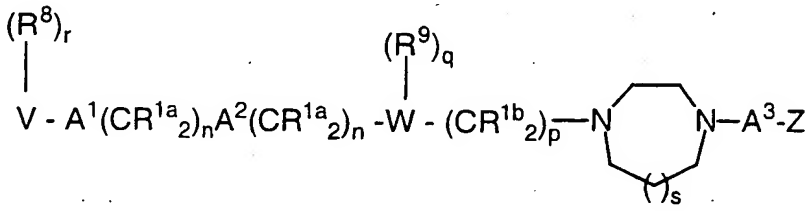
It is also the object of the present invention to provide a compound which is efficacious in vivo as an inhibitor of the growth of cancer cells characterized by a mutated K4B-Ras protein.

A composition which comprises such an inhibitor compound is used in the present invention to treat cancer.

SUMMARY OF THE INVENTION

The present invention comprises piperazine-containing compounds which inhibit prenyl-protein transferases, particularly geranylgeranyl-protein transferase type I. Further contained in this invention are chemotherapeutic compositions containing these prenyl transferase inhibitors and methods for their production.

The compounds of this invention are illustrated by the formula A:

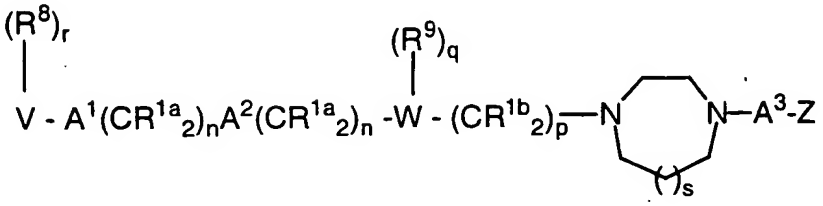


A

DETAILED DESCRIPTION OF THE INVENTION

The compounds of this invention are useful in the inhibition of prenyl-protein transferases and the prenylation of the oncogene protein Ras. In a first

embodiment of this invention, the inhibitors of prenyl-protein transferases are illustrated by the formula A:



A

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-(NR^{10})-, CN, NO_2, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,

c) unsubstituted or substituted C_1-C_6 alkyl wherein the substituent on the substituted C_1-C_6 alkyl is selected from unsubstituted or substituted aryl, heterocyclic, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, and $R^{11}OC(O)$-NR^{10}-;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, NO_2, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, cyanophenyl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NH$-,

$(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{10}OC(O)NH$-;

R^9 is selected from:

a) hydrogen,

b) alkenyl, alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, NO_2, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, -$NR^{10}C(O)$-, O, -$N(R^{10})$-, -$S(O)_2N(R^{10})$-, -$N(R^{10})S(O)_2$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

e) C_2-C_{20} alkenyl, provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

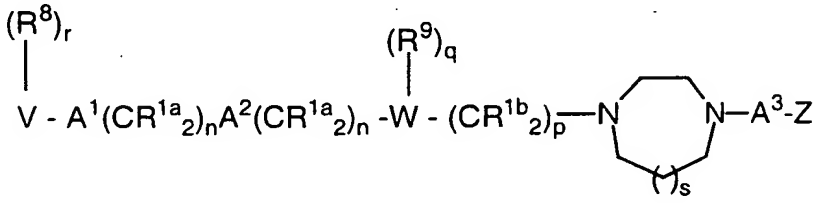
W is a heterocycle;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

	m is	0, 1 or 2;
	n is	0, 1, 2, 3 or 4;
	p is	0, 1, 2, 3 or 4;
	q is	1 or 2;
5	r is	0 to 5, provided that r is 0 when V is hydrogen; and
	s is	0 or 1,

or the pharmaceutically acceptable salts thereof.

10 In a preferred embodiment of this invention, the inhibitors of prenyl-protein transferase are illustrated by the formula A:



A

wherein:

15

R^{1a} is independently selected from: hydrogen or C_1-C_6 alkyl;

R^{1b} is independently selected from:

a) hydrogen,

20 b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,

c) unsubstituted or substituted C_1-C_6 alkyl wherein the substituent on the substituted C_1-C_6 alkyl is selected from unsubstituted or substituted aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$- and -$N(R^{10})_2$;

25 R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^9 is selected from:

a) hydrogen,

b) C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl unsubstituted or substituted by C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, CN, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, $-C(O)NR^{10}$-, O, $-N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

e) C_2-C_{20} alkenyl, and provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

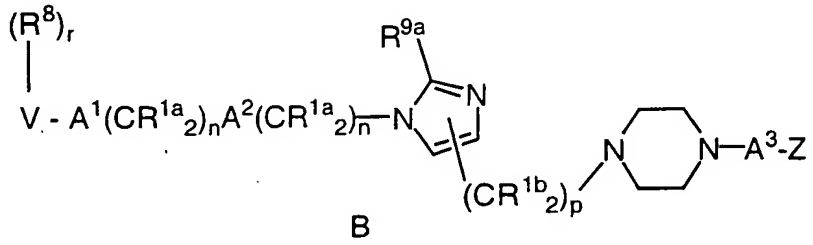
W is a heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, or isoquinolinyl;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is 0, 1, 2, 3 or 4;
q is 1 or 2;
r is 0 to 5, provided that r is 0 when V is hydrogen; and
s is 0 or 1,

or the pharmaceutically acceptable salts thereof.

A preferred embodiment of the compounds of this invention are illustrated by the formula B:



B

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, O, -$N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

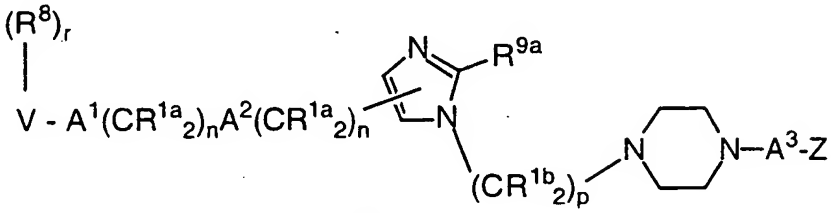
e) C_2-C_{20} alkenyl, and provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is 0, 1, 2, 3 or 4; and
r is 0 to 5, provided that r is 0 when V is hydrogen;

or the pharmaceutically acceptable salts thereof.

Another preferred embodiment of the compounds of this invention are illustrated by the formula C:



C

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,
b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and
c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR^{10}-, O, -N(R^{10})-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

e) C_2-C_{20} alkenyl, and

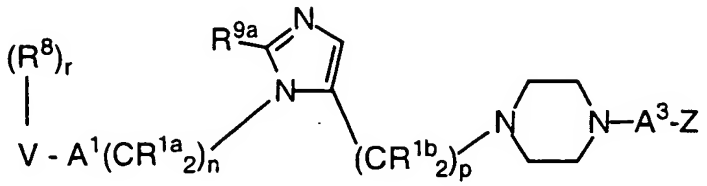
provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is	0, 1 or 2;
n is	0, 1, 2, 3 or 4;
p is	2, 3 or 4; and
r is	0 to 5, provided that r is 0 when V is hydrogen;

or the pharmaceutically acceptable salts thereof.

A further embodiment of the compounds of this invention is illustrated by the formula D:



D

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 is selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR^{10}-, O, -N(R^{10})-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) heterocycle selected from pyridinyl and quinolinyl, and

b) aryl;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;

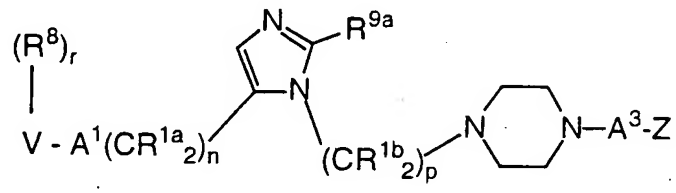
n is 0, 1, 2, 3 or 4;

p is 0, 1, 2, 3 or 4; and

r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

Another embodiment of the compounds of this invention is illustrated by the formula E:



E

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -N(R^{10})$_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 is selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, O, -$N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) heterocycle selected from pyridinyl and quinolinyl, and

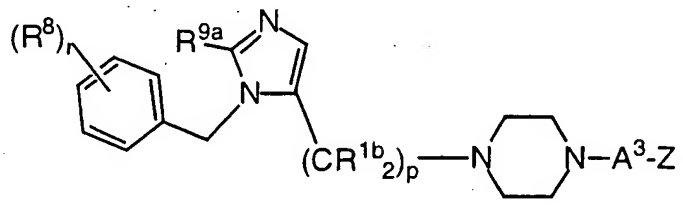
b) aryl;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is	0, 1 or 2;
n is	0, 1, 2, 3 or 4;
p is	2, 3 or 4; and
r is	0 to 5,

or the pharmaceutically acceptable salts thereof.

A still further embodiment of the compounds of this invention is illustrated by the formula F:



F

wherein:

R^{1b} is independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

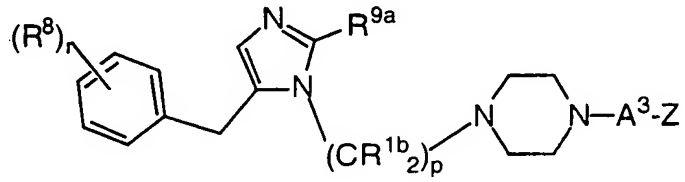
A^3 is -C(O)-;

Z is unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted 2,3-dihydrobenzofuran, unsubstituted or substituted quinoline or unsubstituted or substituted isoquinoline;

p is 1, 2 or 3; and
r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

Another further embodiment of the compounds of this invention is illustrated by the formula G:



G

wherein:

R^{1b} is independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^3 is -C(O)-;

Z is unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted 2,3-dihydrobenzofuran, unsubstituted or substituted quinoline or unsubstituted or substituted isoquinoline;

p is 2 or 3; and

r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

Specific examples of the compounds of this invention are as follows:

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-methoxyquinolin-4-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-diethylamino-3-ethoxypyrid-5-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-ethylamino-4-isoquinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-bromo-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(pent-1-ynyl)-1-naphthoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(prop-1-ynyl)-1-naphthoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-propyl-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-bromo-3-methylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl -4-(prop-1-ynyl)benzoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methyl-4-pentylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-cyclopropyleth-ynyl-5-methoxybenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-methoxy-2-pent-1-ynylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethynylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-indoloyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,5-dimethylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(8-quinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-ethoxy-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-quinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methoxy-4-methylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-isoquinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2,3-dihydrobenzofuran-7-oyl)piperazine

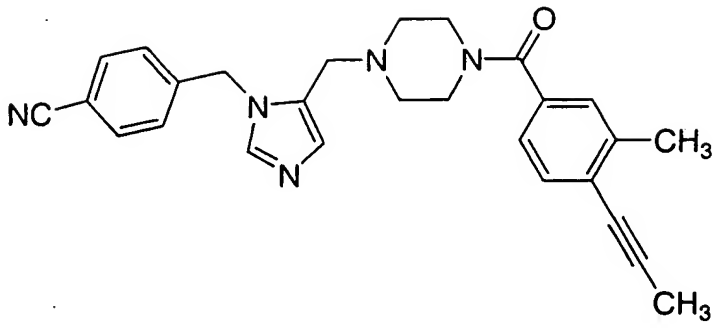
4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,4-dimethylbenzoyl) piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-naphthoyl)piperazine

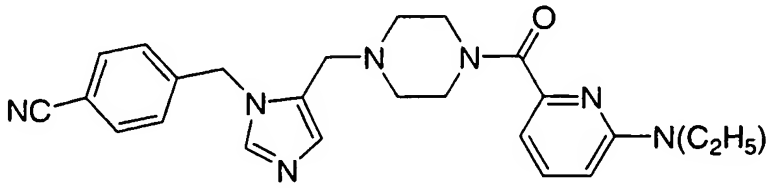
or a pharmaceutically acceptable salt or optical isomer thereof.

Specific compounds of this invention are as follows:

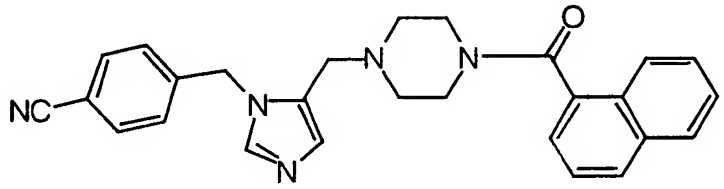
4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl -4-(prop-1-ynyl)benzoyl]piperazine



4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine



4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-naphthoyl)piperazine



or the pharmaceutically acceptable salts or optical isomers thereof.

The compounds of the instant invention differ from previously disclosed piperazinone-containing and piperazine-containing compounds, (PCT Publ. No. WO 96/30343 - October 3, 1996; PCT Publ. No. WO 96/31501 - October 10, 1996; PCT Publ. No. WO 97/36593 - October 9, 1997; PCT Publ. No. WO 97/36592 - October 9, 1997) that were described as inhibitors of farnesyl-protein transferase (FPTase), in that, among other things, the instant compounds are dual inhibitors of farnesyl-protein transferase and geranylgeranyl-protein transferase type I (GGTase-I). The compounds are further characterized in that the inhibitory activity of the compounds against GGTase-I is greater than the inhibitory activity against FPTase.

Preferably, the compounds of the instant invention inhibit FPTase in vitro (Example 28) at an IC_{50} of less than 1 μM and inhibit GGTase-I in vitro (Example 29) at an IC_{50} of less than 50 nM. Preferably, the ratio of the IC_{50} of the compounds of the instant invention for in vitro inhibition of FPTase to the IC_{50} of the compounds of the instant invention for in vitro inhibition of GGTase type I is greater than 5. Also preferably, the compounds of the instant invention inhibit the cellular processing of the Rap1 protein (Example 34) at an EC_{50} of less than about 1 μM. More preferably, the compounds of the instant invention inhibit the cellular processing of the Rap1 protein (Example 34) at an EC_{50} of less than about 50 nM. Also more preferably, the ratio of the IC_{50} of the compounds of the instant invention for in vitro inhibition of FPTase to the IC_{50} of the compounds of the instant invention for in vitro inhibition of GGTase type I is greater than 25. Also more preferably, the ratio of the EC_{50} of the compounds of the instant invention for inhibition of the cellular processing of the hDJ protein (Example 33) to the EC_{50} of the compounds of the instant invention for inhibition of the cellular processing of the Rap1 protein is about equal to or less than 1.

The compounds of the present invention may have asymmetric centers and occur as racemates, racemic mixtures, and as individual diastereomers, with all possible isomers, including optical isomers, being included in the present invention. When any variable (e.g. aryl, heterocycle, R^1, R^2 etc.) occurs more than one time in any constituent, its definition on each occurrence is independent at every other occurrence. Also, combinations of substituents/or variables are permissible only if such combinations result in stable compounds.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms; "alkoxy" represents an alkyl group of indicated number of carbon atoms attached through an oxygen bridge. "Halogen" or "halo" as used herein means fluoro, chloro, bromo and iodo.

As used herein, "cycloalkyl" is intended to include monocyclic saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. Examples of such cycloalkyl groups includes, but are not limited to, cyclopropyl, cyclobutyl, cyclohexyl, cycloheptyl and cyclooctyl.

As used herein, "aryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic. Examples of such aryl elements include phenyl, naphthyl, tetrahydronaphthyl, indanyl, biphenyl, phenanthryl, anthryl or acenaphthyl.

The term heterocycle or heterocyclic, as used herein, represents a stable 5- to 7-membered monocyclic or stable 8- to 11-membered bicyclic heterocyclic ring which is either saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from the group consisting of N, O, and S, and including any bicyclic group in which any of the above-defined heterocyclic rings are fused to a benzene ring. The term heterocycle or heterocyclic includes heteroaryl moieties. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic elements include, but are not limited to, azepinyl, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydro-benzothiopyranyl sulfone, furyl, imidazolidinyl, imidazolinyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isothiazolidinyl, morpholinyl, naphthyridinyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, 2-oxopiperazinyl, 2-oxopiperdinyl, 2-oxopyrrolidinyl, piperidyl, piperazinyl, pyridyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydrofuryl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiazolyl, thiazolinyl, thienofuryl, thienothienyl, and thienyl.

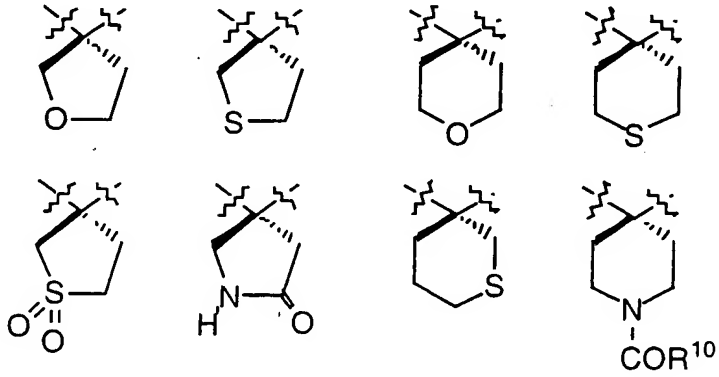
As used herein, "heteroaryl" is intended to mean any stable monocyclic or bicyclic carbon ring of up to 7 members in each ring, wherein at least one ring is aromatic and wherein from one to four carbon atoms are replaced by heteroatoms selected from the group consisting of N, O, and S. Examples of such heterocyclic elements include, but are not limited to, benzimidazolyl, benzisoxazolyl, benzofurazanyl, benzopyranyl, benzothiopyranyl, benzofuryl, benzothiazolyl, benzothienyl, benzoxazolyl, chromanyl, cinnolinyl, dihydrobenzofuryl, dihydrobenzothienyl, dihydrobenzothiopyranyl, dihydrobenzothiopyranyl sulfone, furyl, imidazolyl, indolinyl, indolyl, isochromanyl, isoindolinyl, isoquinolinyl, isothiazolyl, naphthyridinyl, oxadiazolyl, pyridyl, pyrazinyl, pyrazolyl, pyridazinyl, pyrimidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, tetrahydroisoquinolinyl, tetrahydroquinolinyl, thiazolyl, thienofuryl, thienothienyl, and thienyl.

As used herein in the definition of Z the substituted aryl and substituted heteroaryl include moieties containing from 1 to 3 substituents in addition to the point of attachment to the rest of the compound. Preferably, such substituents

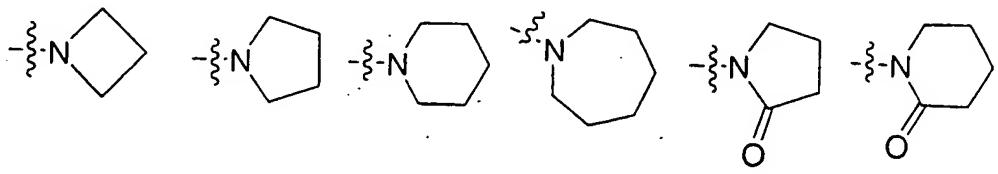
are selected from the group which includes but is not limited to F, Cl, Br, CF_3, OCF_3, NH_2, $N(C_1\text{-}C_6$ alkyl$)_2$, NO_2, SO_2CH_3, CN, $(C_1\text{-}C_6$ alkyl$)O$-, (aryl)O-, -OH, $(C_1\text{-}C_6$ alkyl$)S(O)_m$-, $(C_1\text{-}C_6$ alkyl$)C(O)NH$-, $H_2N\text{-}C(NH)$-, $(C_1\text{-}C_6$ alkyl$)C(O)$-, $(C_1\text{-}C_6$ alkyl$)OC(O)$-, N_3,$(C_1\text{-}C_6$ alkyl$)OC(O)NH$-, phenyl, pyridyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thienyl, furyl, isothiazolyl and $C_1\text{-}C_{20}$ alkyl, $C_2\text{-}C_6$ alkenyl.

When R^2 and R^3 are combined to form $-(CH_2)_u$-, cyclic moieties are formed. Examples of such cyclic moieties include, but are not limited to:

In addition, such cyclic moieties may optionally include a heteroatom(s). Examples of such heteroatom-containing cyclic moieties include, but are not limited to:



The moiety formed when, in the definition of R^6, R^7 and R^{7a}, R^6 and R^7 or R^7 and R^{7a} are joined to form a ring, is illustrated by, but not limited to, the following:



Lines drawn into the ring systems from substituents (such as from R^{1a}, R^{1b}, R^8 etc.) indicate that the indicated bond

may be attached to any of the substitutable ring carbon atoms.

Preferably, R^{1a} and R^{1b} are independently selected from: hydrogen, -$N(R^{10})_2$, $R^{10}C(O)NR^{10}$- or unsubstituted or substituted C_1-C_6 alkyl wherein the substituent on the substituted C_1-C_6 alkyl is selected from unsubstituted or substituted phenyl, -$N(R^{10})_2$, $R^{10}O$- and $R^{10}C(O)NR^{10}$-.

Preferably, R^9 is hydrogen, chloro or C_1-C_6 alkyl.

Preferably, R^{10} is selected from H, C_1-C_6 alkyl, benzyl and aryl.

Preferably, A^1 and A^2 are independently selected from: a bond, -$C(O)NR^{10}$-, -$NR^{10}C(O)$-, O, -$N(R^{10})$-, -$S(O)_2N(R^{10})$- and -$N(R^{10})S(O)_2$-. Most preferably, A^1 and A^2 are a bond.

Preferably, A^3 is -C(O)-.

Preferably, V is selected from heteroaryl and aryl. More preferably, V is phenyl.

Preferably, W is selected from imidazolyl, pyridinyl, thiazolyl, indolyl, quinolinyl, and isoquinolinyl. More preferably W is selected from imidazolyl and pyridinyl.

Preferably, Z is selected from unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted quinoline, unsubstituted or substituted isoquinoline and unsubstituted or substituted 2,3-dihydrobenzofuran, wherein the substituted phenyl, substituted napthyl, substituted pyridyl, substituted quinoline, substituted isoquinoline and substituted 2,3-dihydrobenzofuran, are substituted with one or more of the following:

a) OH,
b) alkoxy,
c) aryloxy,
d) C_1-C_6 alkyl,
e) NO_2,
f) halogen,
g) C_2-C_6 alkenyl,
h) OCF_3,
i) SO_2CH_3, or
j) (C_1-C_6 alkyl)C(O)NH-

More preferably, Z is unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted isoquinoline or unsubstituted or substituted 2,3-dihydrobenzofuran, wherein the substituted phenyl, substituted napthyl, substituted isoquinoline and substituted 2,3-dihydrobenzofuran, is substituted with one or more of the following:

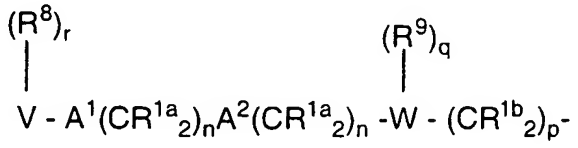
a) OH,
b) alkoxy,
c) aryloxy,
d) C_1-C_6 alkyl,
e) NO_2,
f) halogen,
g) C_2-C_6 alkenyl,
h) OCF_3,
i) SO_2CH_3, or
j) (C_1-C_6 alkyl)C(O)NH-

Preferably, n and r are independently 0, 1, or 2.

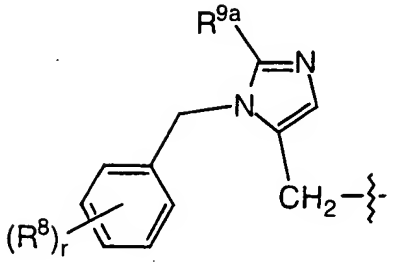
Preferably p is 1, 2 or 3.

Preferably s is 0.

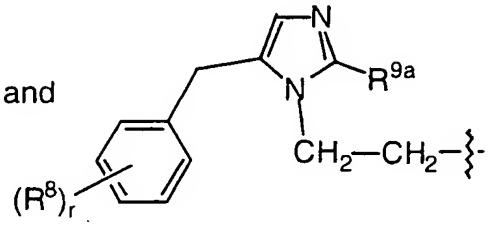
Preferably, the moiety



is selected from:



and



Preferably, the moiety

$$- A^1(CR^{1a}{}_2)_nA^2(CR^{1a}{}_2)_n -$$

is not a bond.

It is intended that the definition of any substituent or variable (e.g., R^{1a}, R^9, n, etc.) at a particular location in a molecule be independent of its definitions elsewhere in that molecule. Thus, $-N(R^{10})_2$ represents -NHH, $-NHCH_3$, $-NHC_2H_5$, etc. It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials.

The pharmaceutically acceptable salts of the compounds of this invention include the conventional non-toxic salts of the compounds of this invention as formed, e.g., from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like: and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, trifluoroacetic and the like.

The pharmaceutically acceptable salts of the compounds of this invention can be synthesized from the compounds of this invention which contain a basic moiety by conventional chemical methods. Generally, the salts are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents.

Reactions used to generate the compounds of this invention are prepared by employing reactions as shown in the Schemes 1-14, in addition to other standard manipulations such as ester hydrolysis, cleavage of protecting groups, etc., as may be known in the literature or exemplified in the experimental procedures. The point of attachment to the ring of substituents (i.e., R^8), as shown in the Schemes, is illustrative only and is not meant to be limiting. Substituent Z', as shown in the

Schemes, represents the substituent Z as defined hereinabove or a protected precursor thereof.

These reactions may be employed in a linear sequence to provide the compounds of the invention or they may be used to synthesize fragments which are subsequently joined by the alkylation reactions described in the Schemes.

Synopsis of Schemes 1-14:

The requisite intermediates are in some cases commercially available, or can be prepared according to literature procedures, for the most part. In Scheme 1, for example, boc-protected piperazine VI, available commercially or by procedures known to those skilled in the art, can be coupled to suitable substituted carboxylic acids using a variety of dehydrating agents such as DCC (dicyclohexycarbodiimide) or EDC·HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) in a solvent such as methylene chloride, chloroform, dichloroethane, or dimethylformamide. The product VII is then deprotected with acid, for example hydrogen chloride in chloroform or ethyl acetate, or trifluoroacetic acid in methylene chloride to give intermediate VIII. Intermediate VIII can itself be reductively alkylated with a variety of aldehydes, such as IX. The aldehydes can be prepared by standard procedures, such as that described by O. P. Goel, U. Krolls, M. Stier and S. Kesten in Organic Syntheses, 1988, *67*, 69-75, from the appropriate amino acid (Scheme 2). The reductive alkylation can be accomplished at pH 5-7 with a variety of reducing agents, such as sodium triacetoxyborohydride or sodium cyanoborohydride in a solvent such as dichloroethane, methanol or dimethylformamide. The product X can be deprotected to give the final compounds XI with trifluoroacetic acid in methylene chloride. The final product XI is isolated in the salt form, for example, as a trifluoroacetate, hydrochloride or acetate salt, among others. The product diamine XI can further be selectively protected to obtain XII, which can subsequently be reductively alkylated with a second aldehyde to obtain XIII. Removal of the protecting group, and conversion to cyclized products such as the dihydroimidazole XV can be accomplished by literature procedures.

As shown in Scheme 3, the piperazine intermediate VIII can be reductively alkylated with other aldehydes such as 1-trityl-4-imidazolyl-carboxaldehyde or 1-trityl-4-imidazolylacetaldehyde, to give products such as XVI. The trityl protecting group can be removed from XVI to give XVII, or alternatively, XVI can first be treated with an alkyl halide then subsequently deprotected to give the

alkylated imidazole XVIII. Alternatively, the intermediate VIII can be acylated or sulfonylated by standard techniques.

Scheme 4 illustrates the incorporation of an indole moiety for the substituent W in place of the preferred benzylimidazolyl moiety.

Scheme 5 illustrates synthesis of an instant compound wherein a non-hydrogen R^{9b} is incorporated in the instant compound. Thus, a readily available 4-substituted imidazole XXVI may be selectively iodinated to provide the 5-iodoimidazole XXVII. That imidazole may then be protected and coupled to a suitably substituted benzyl moiety to provide intermediate XXVIII. Attachment of the imidazolyl nitrogen via an ethyl linker to the piperazine nitrogen of intermediate VIII, described above, provides the instant compound XXIX.

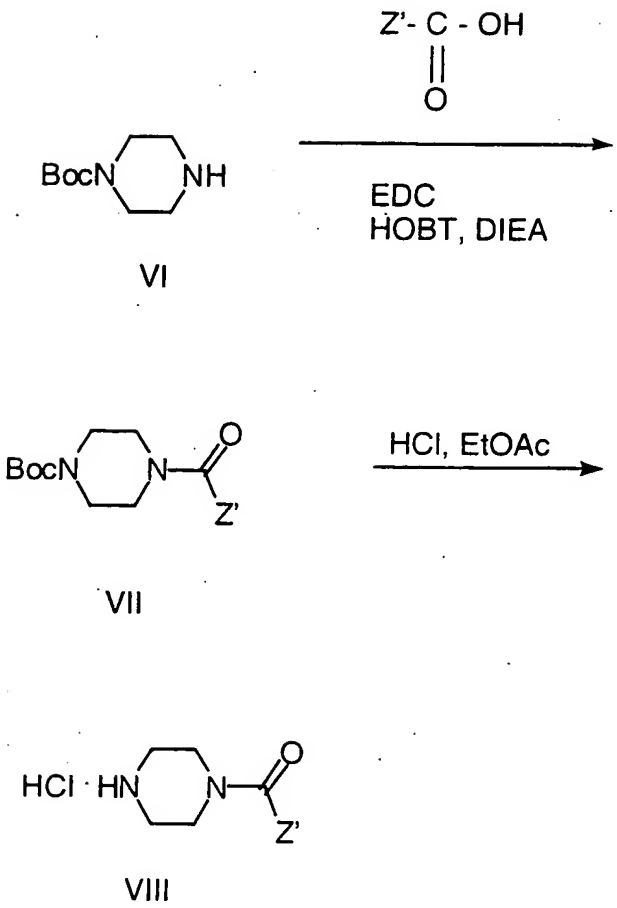
Compounds of the instant invention wherein the $A^1(CR^{1a}{}_2)_nA^2(CR^{1a}{}_2)_n$ linker is oxygen may be synthesized by methods known in the art, for example as shown in Scheme 6. The suitably substituted phenol XXX may be reacted with methyl N-(cyano)methanimidate to provide the 4-phenoxyimidazole XXXI. After selective protection of one of the imidazolyl nitrogens, the intermediate XXXII can undergo alkylation reactions.

If the piperazine VIII is reductively alkylated with an aldehyde which also has a protected hydroxyl group, such as XXXIII in Scheme 7, the protecting groups can be subsequently removed to unmask the hydroxyl group. The Boc protected amino alcohol XXXIV can then be utilized to synthesize 2-aziridinylmethylpiperazines such as XXXV.

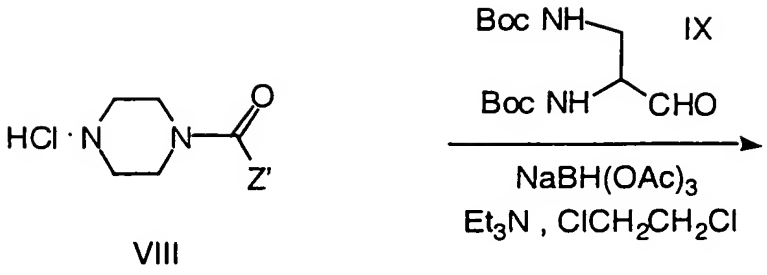
Schemes 8-12 illustrate syntheses of suitably substituted aldehydes useful in the syntheses of the instant compounds wherein the variable W is present as a pyridyl moiety. Similar synthetic strategies for preparing alkanols that incorporate other heterocyclic moieties for variable W are also well known in the art. For example, Scheme 12 illustrates the preparation of the corresponding quinoline aldehyde.

Scheme 13 depicts a general method for synthesizing a key intermediate useful in the preparation of preferred embodiments of the instant invention wherein V is phenyl and W is imidazole. A piperazine moiety can be readily added to this benzyl-imidazole intermediate as set forth in Scheme 14.

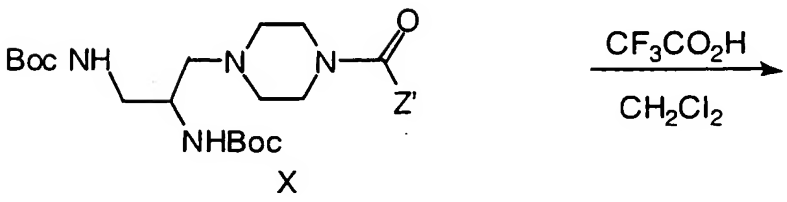
SCHEME 1


Z'- C - OH
O
BocN
NH
EDC
HOBT, DIEA
VI
BocN
N
O
Z'
HCl, EtOAc
VII
HCl · HN
N
O
Z'
VIII

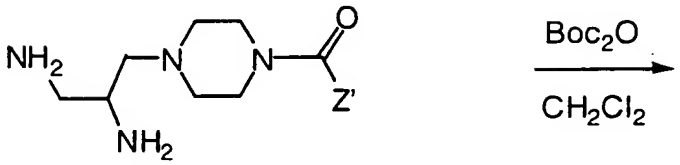
SCHEME 2



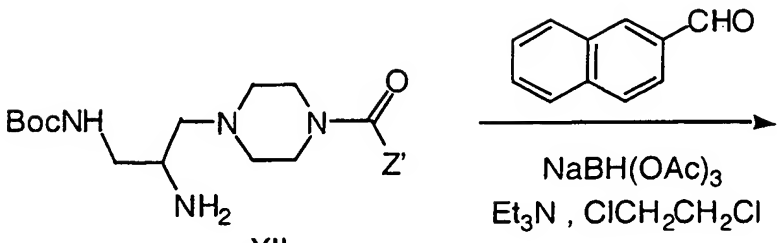
VIII



X

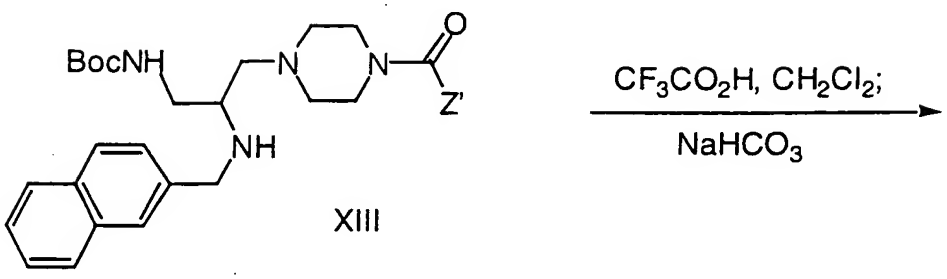


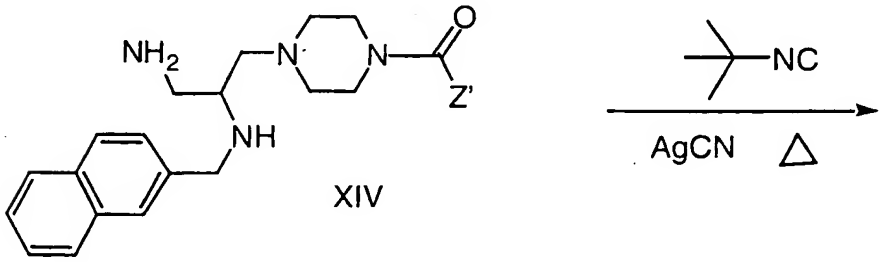
XI

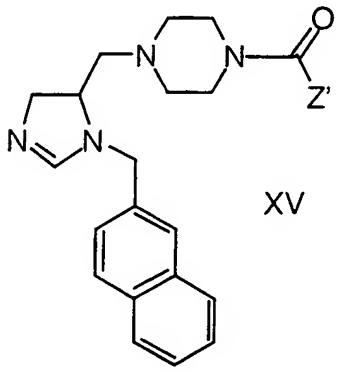


XII

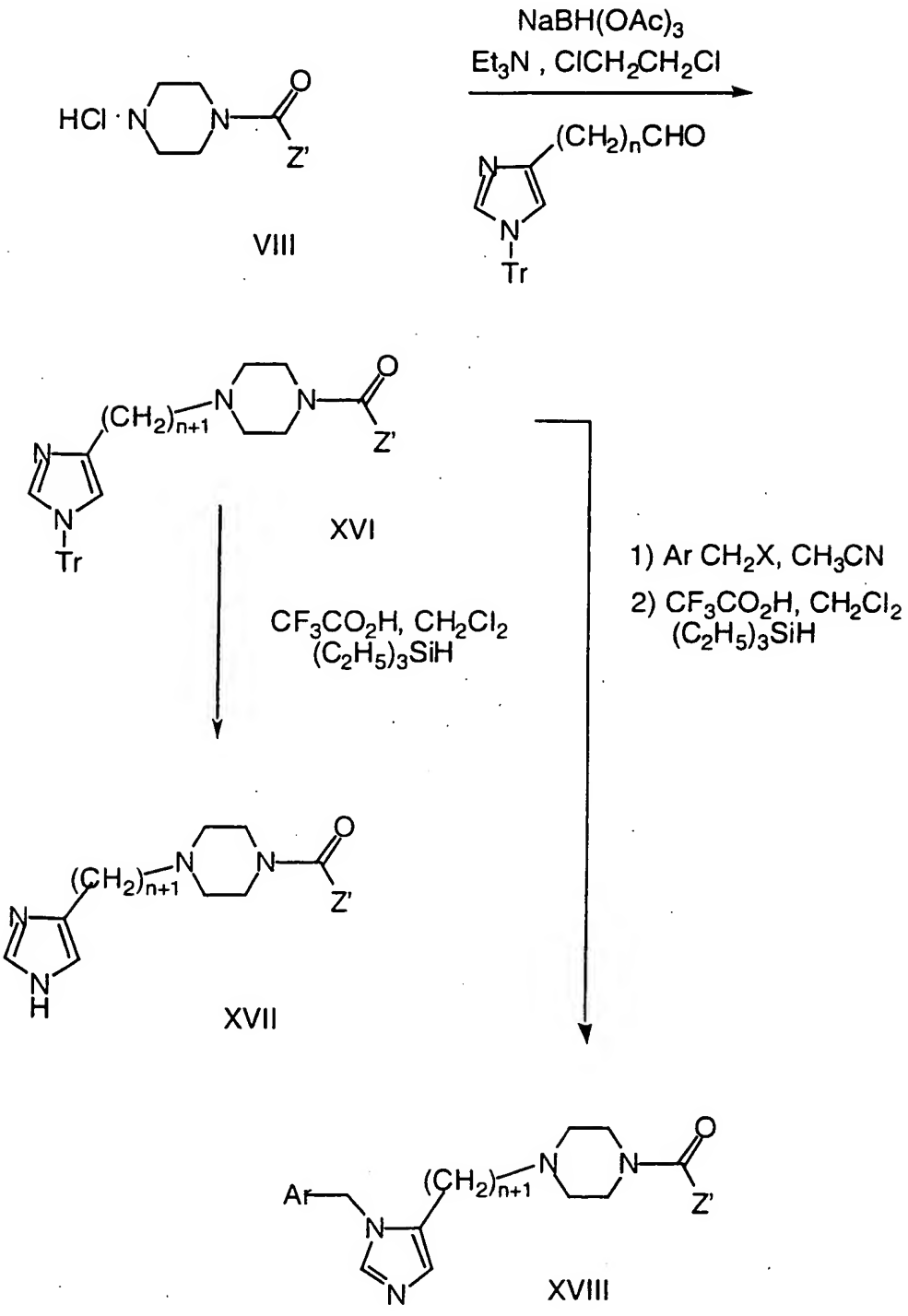
SCHEME 2 (continued)



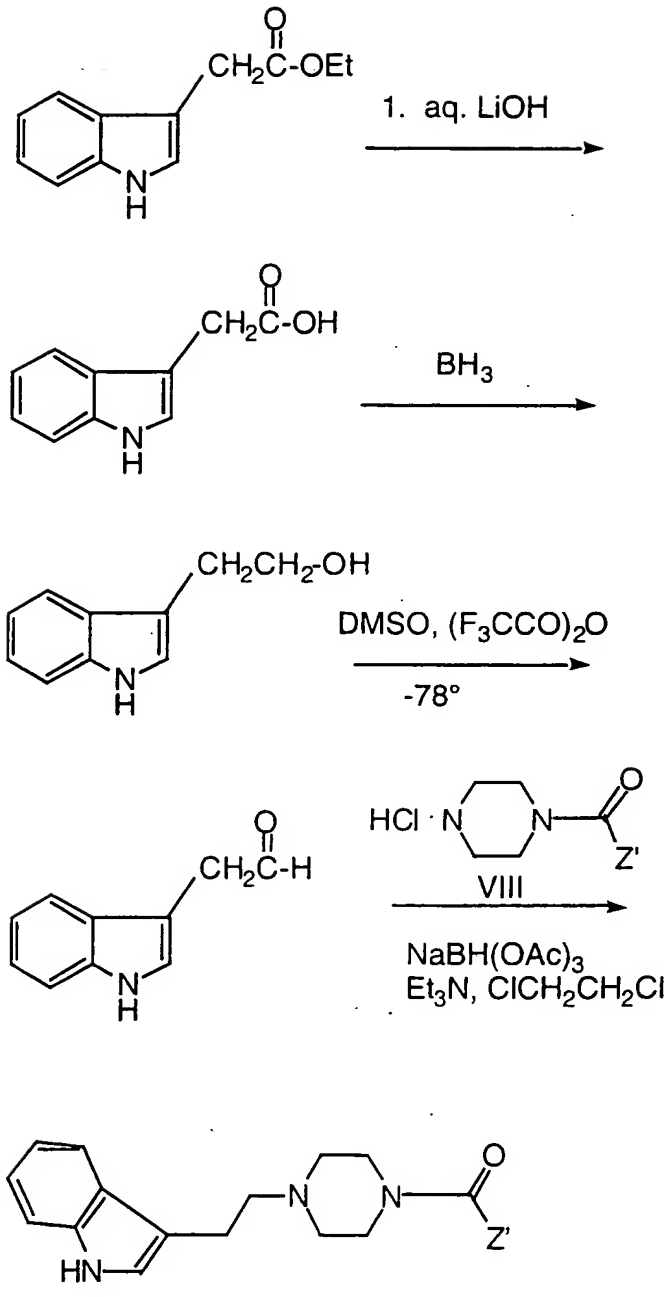




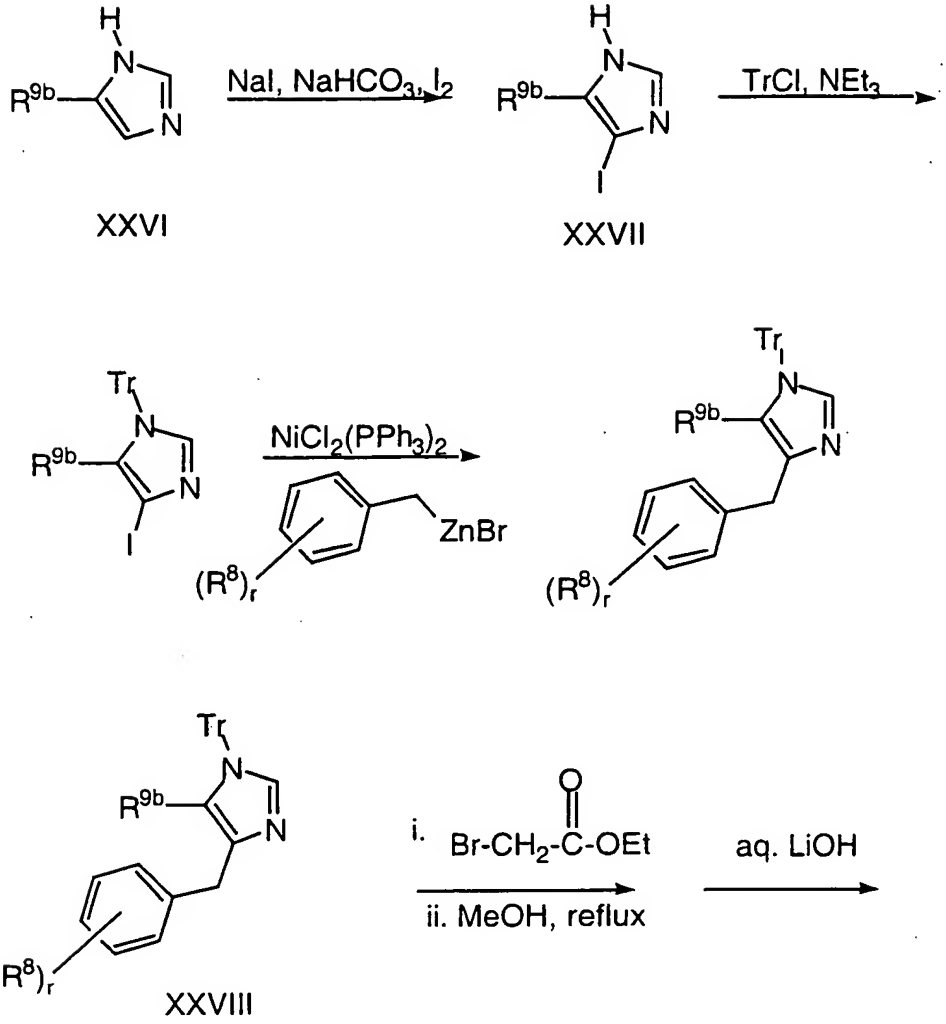
SCHEME 3


NaBH(OAc)3
Et3N , ClCH2CH2Cl
HCl
Z'
VIII
(CH2)nCHO
Tr
(CH2)n+1
XVI
CF3CO2H, CH2Cl2
(C2H5)3SiH
1) Ar CH2X, CH3CN
2) CF3CO2H, CH2Cl2
(C2H5)3SiH
XVII
Ar
XVIII

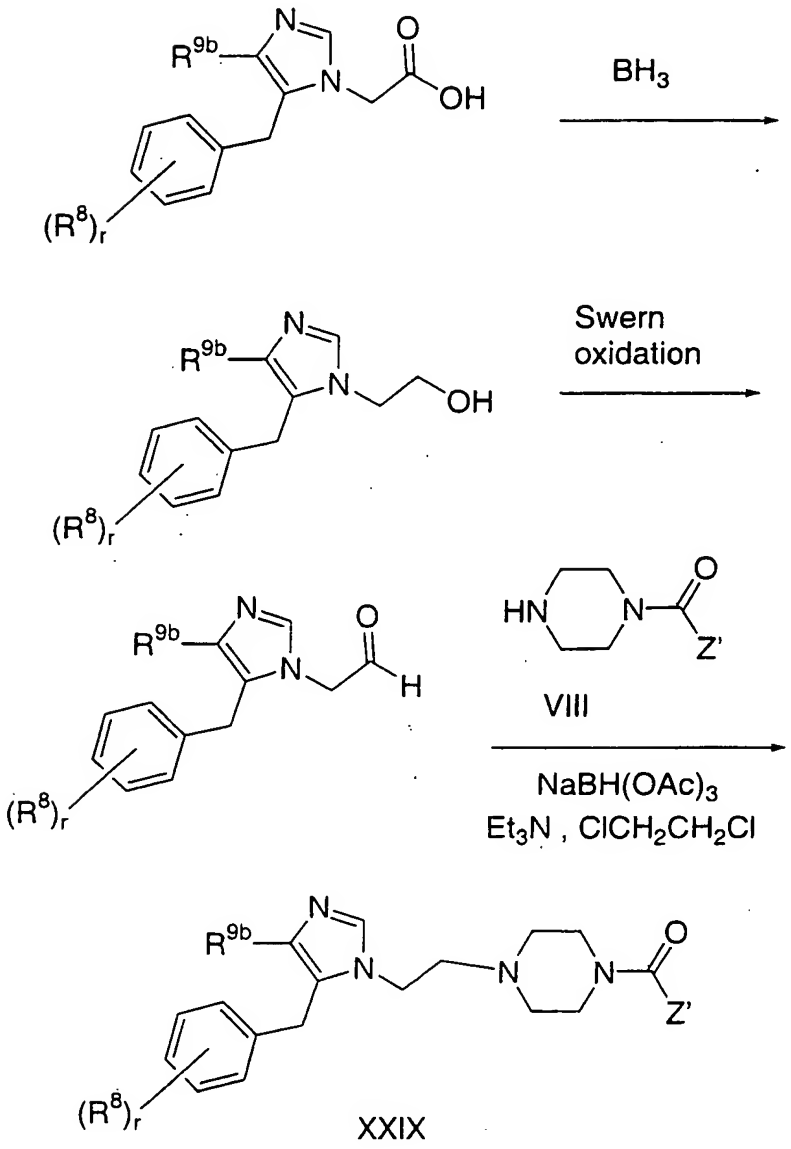
SCHEME 4


CH2C-OEt
1. aq. LiOH
CH2C-OH
BH3
CH2CH2-OH
DMSO, (F3CCO)2O
-78°
CH2C-H
HCl · N
N
Z'
VIII
NaBH(OAc)3
Et3N, ClCH2CH2Cl
HN

SCHEME 5

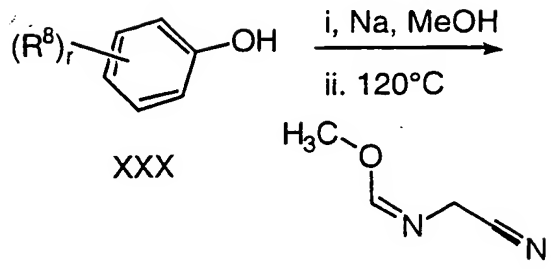

NaI, NaHCO3, I2
TrCl, NEt3
XXVI
XXVII
NiCl2(PPh3)2
ZnBr
i. Br-CH2-C-OEt
ii. MeOH, reflux
aq. LiOH
XXVIII

SCHEME 5 (continued)

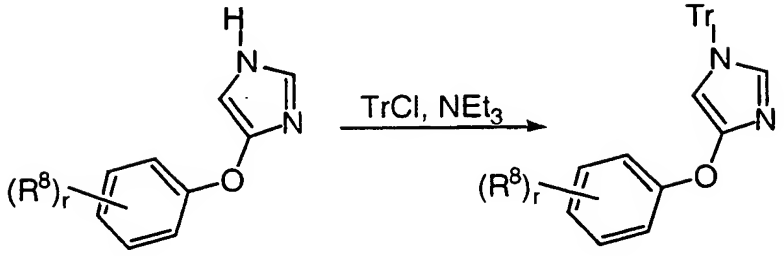


XXIX

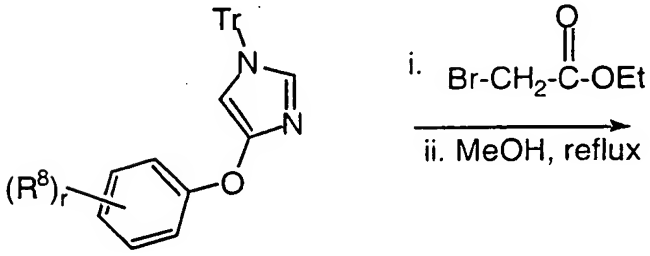
SCHEME 6



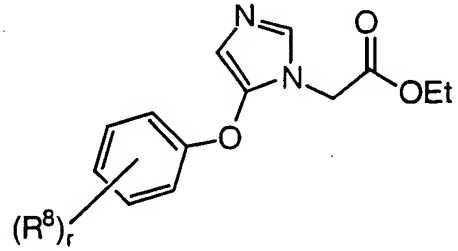
XXX



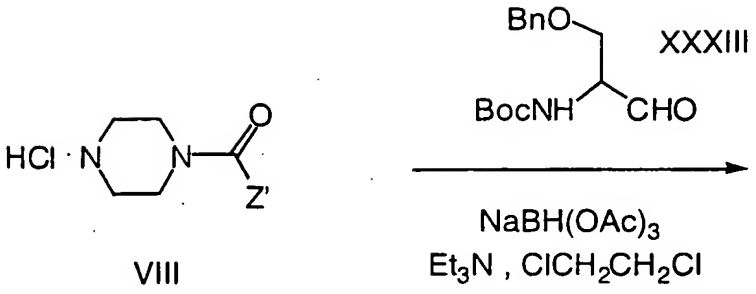
XXXI



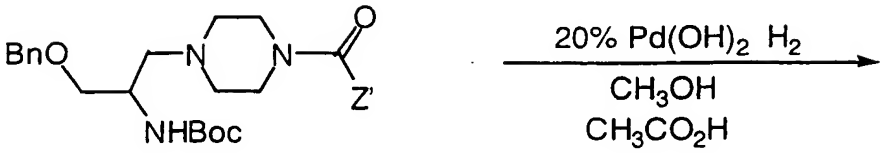
XXXII

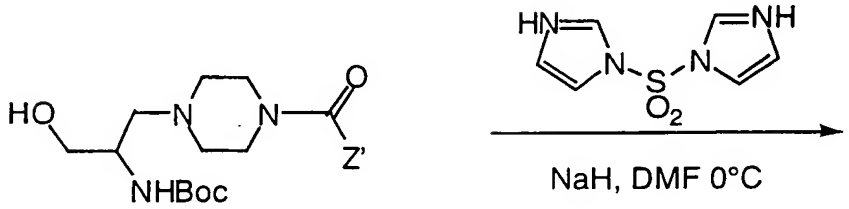


SCHEME 7

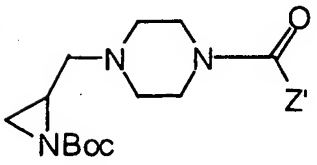


VIII



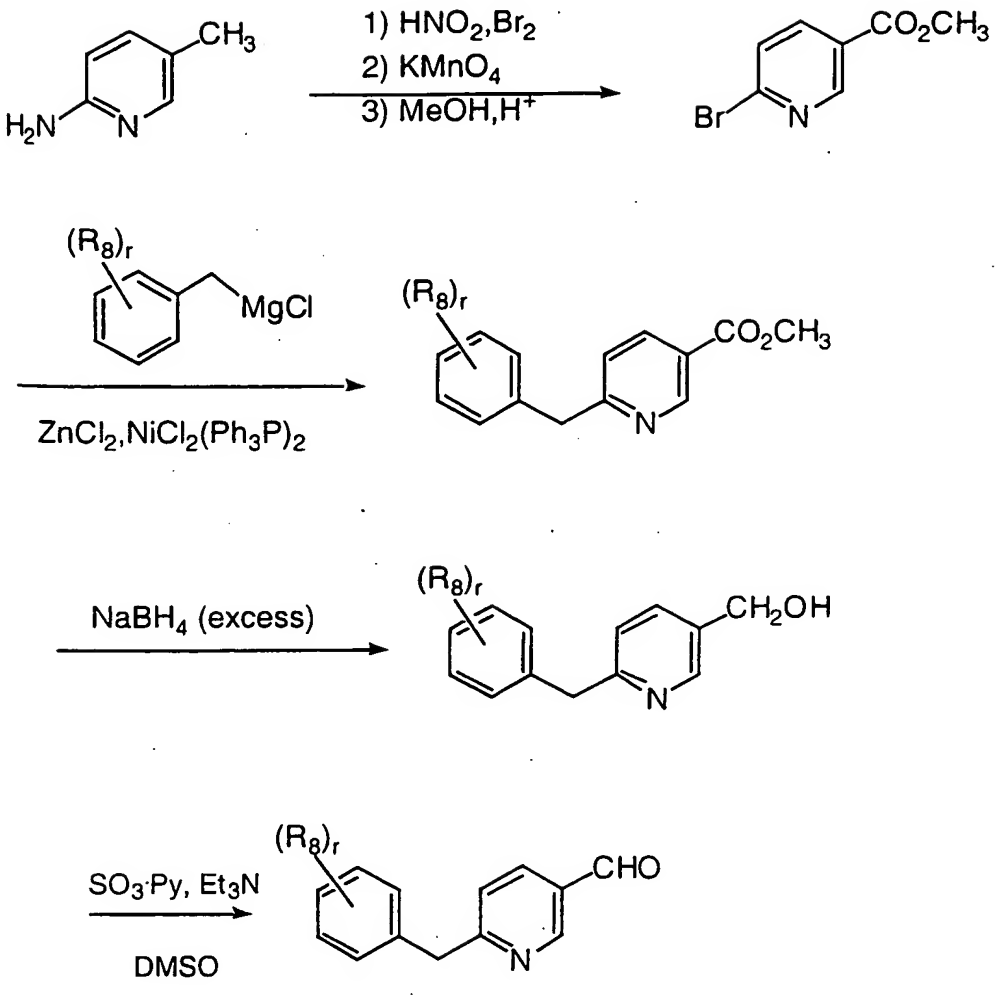


XXXIV

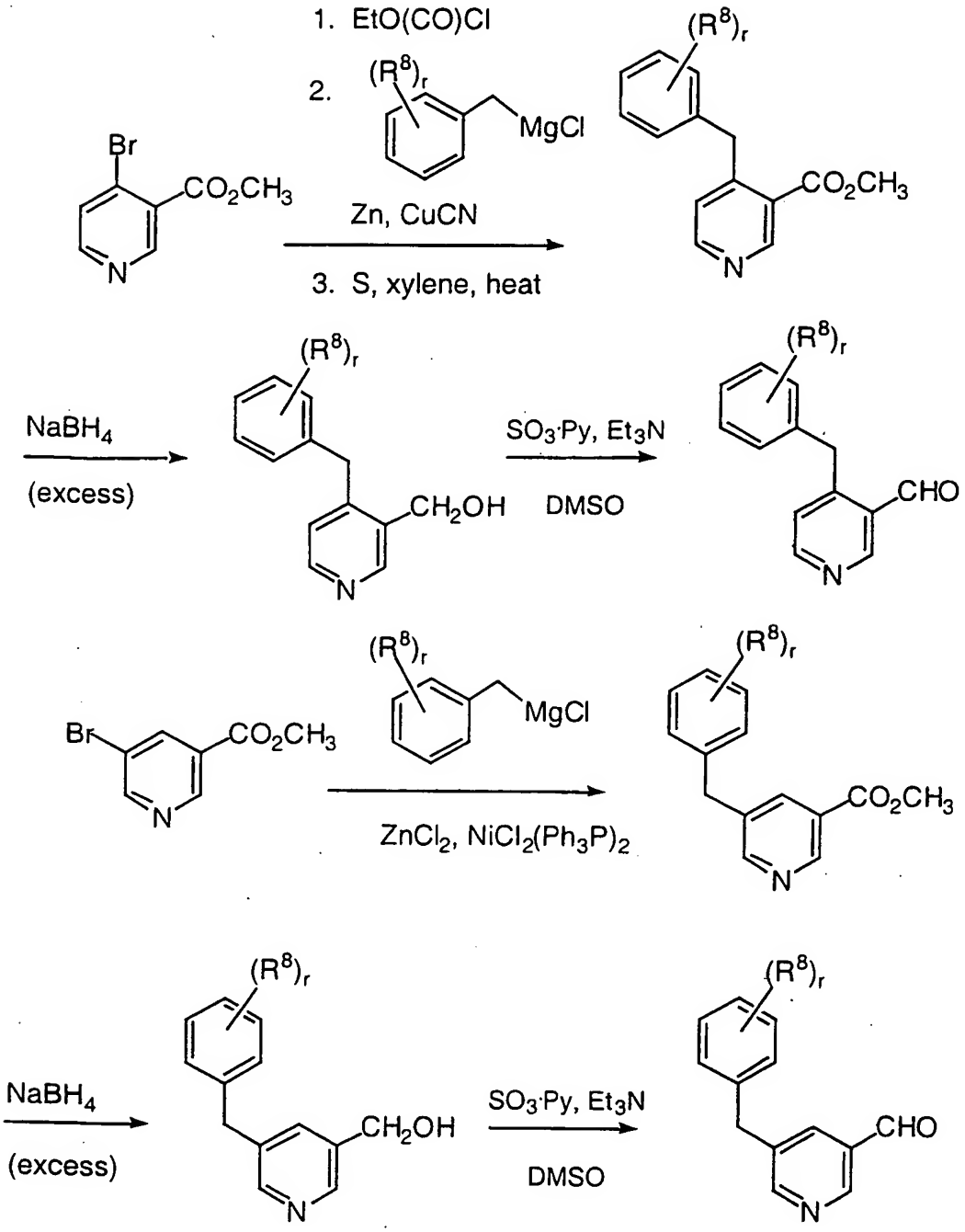


XXXV

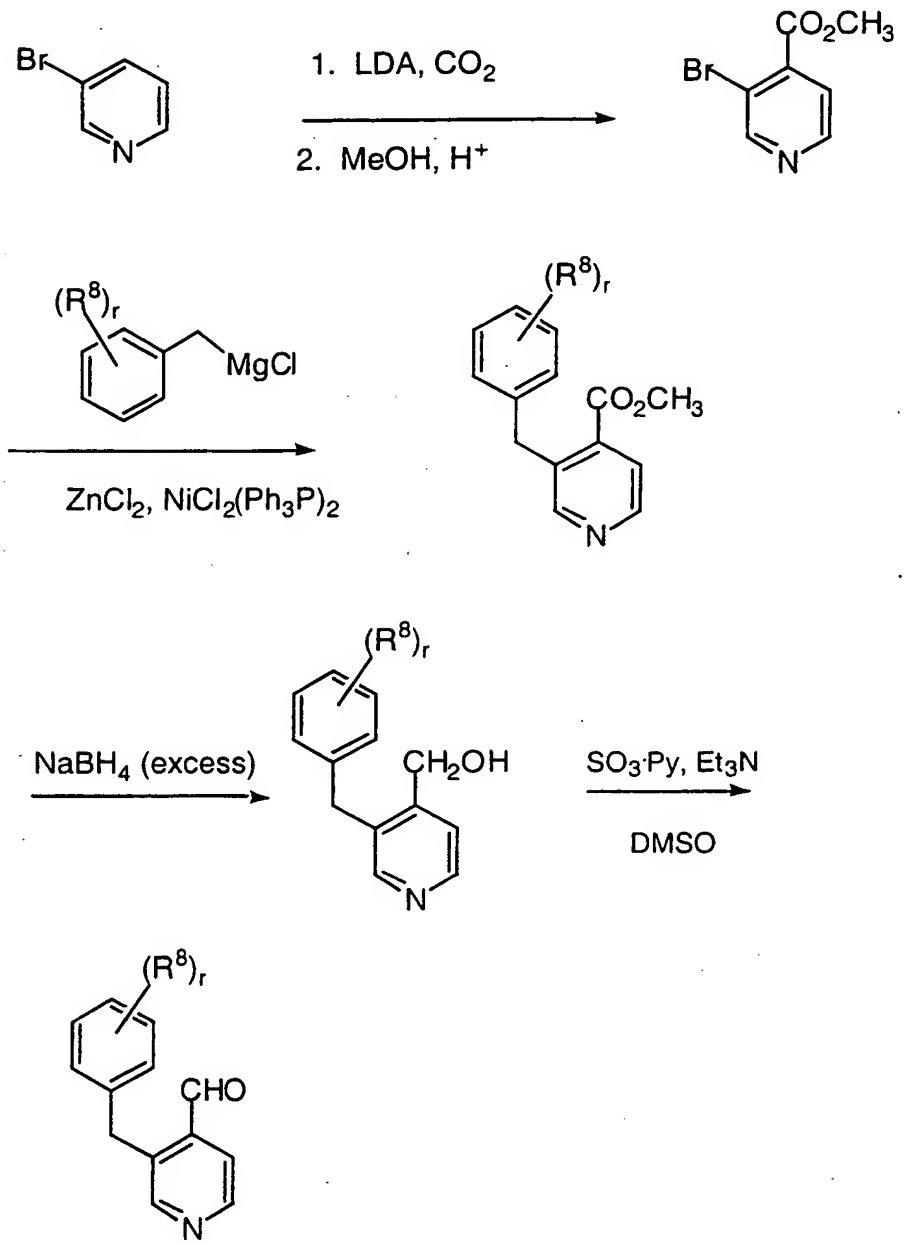
SCHEME 8


1) HNO_2,Br_2
2) $KMnO_4$
3) MeOH,H^+
CH_3
H_2N
N
CO_2CH_3
Br
$(R_8)_r$
MgCl
$ZnCl_2$,$NiCl_2(Ph_3P)_2$
CO_2CH_3
$NaBH_4$ (excess)
CH_2OH
SO_3·Py, Et_3N
DMSO
CHO

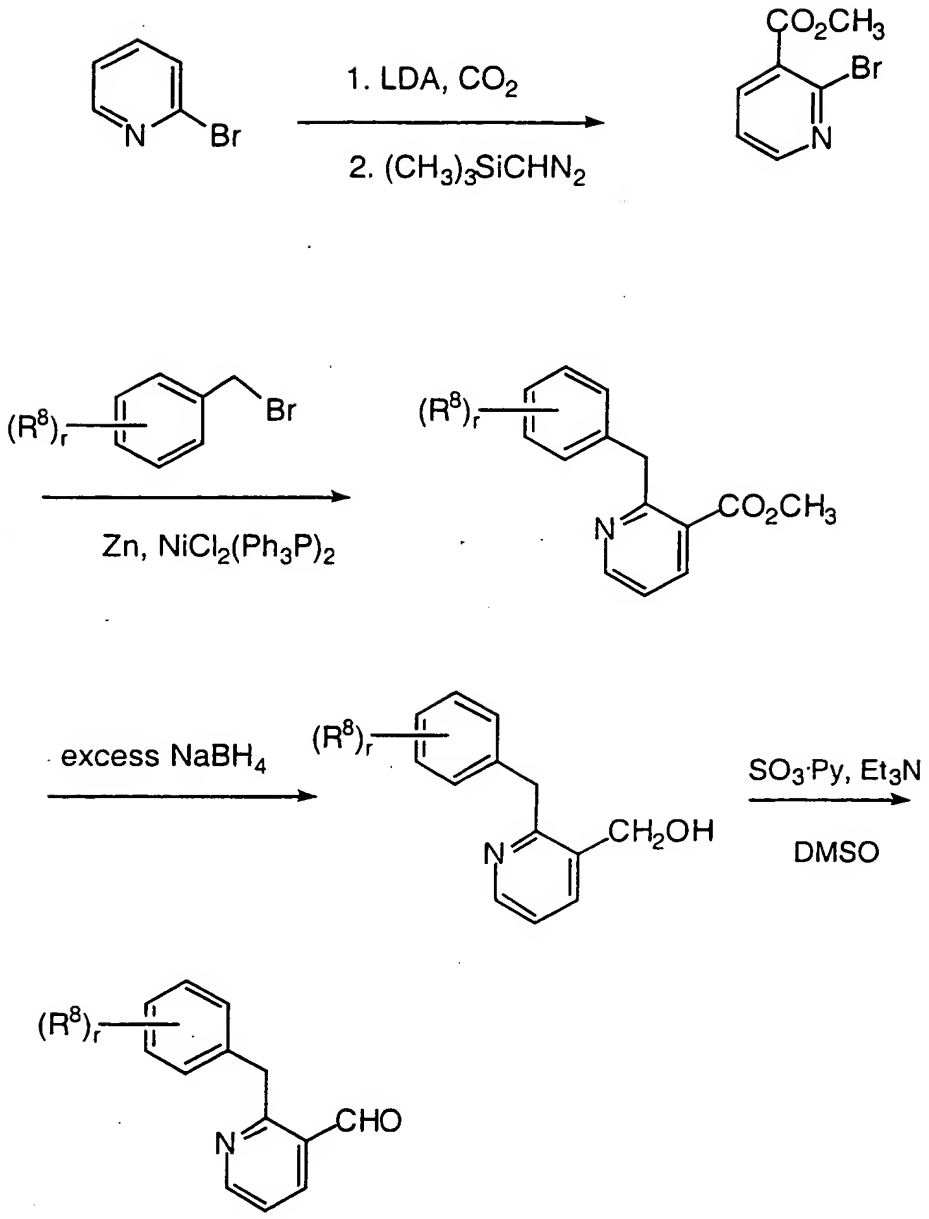
SCHEME 9


1. EtO(CO)Cl
2.
MgCl
Br
CO2CH3
Zn, CuCN
3. S, xylene, heat
(R8)r
N
NaBH4
(excess)
CH2OH
SO3·Py, Et3N
DMSO
CHO
ZnCl2, NiCl2(Ph3P)2

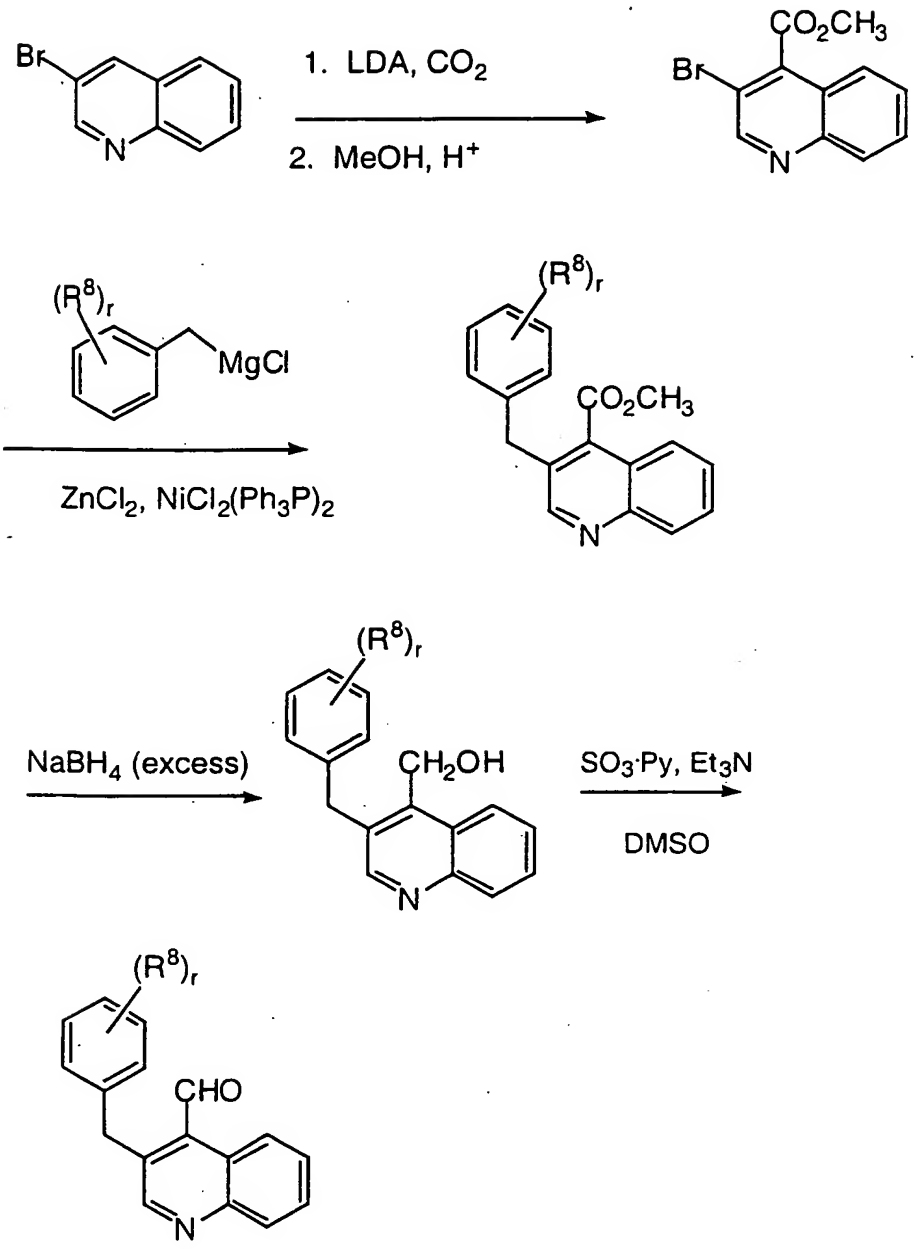
SCHEME 10


Br
N
1. LDA, CO_2
2. MeOH, H^+
CO_2CH_3
Br
N
$(R^8)_r$
MgCl
$ZnCl_2$, $NiCl_2(Ph_3P)_2$
$(R^8)_r$
CO_2CH_3
N
$(R^8)_r$
$NaBH_4$ (excess)
CH_2OH
N
SO_3·Py, Et_3N
DMSO
$(R^8)_r$
CHO
N

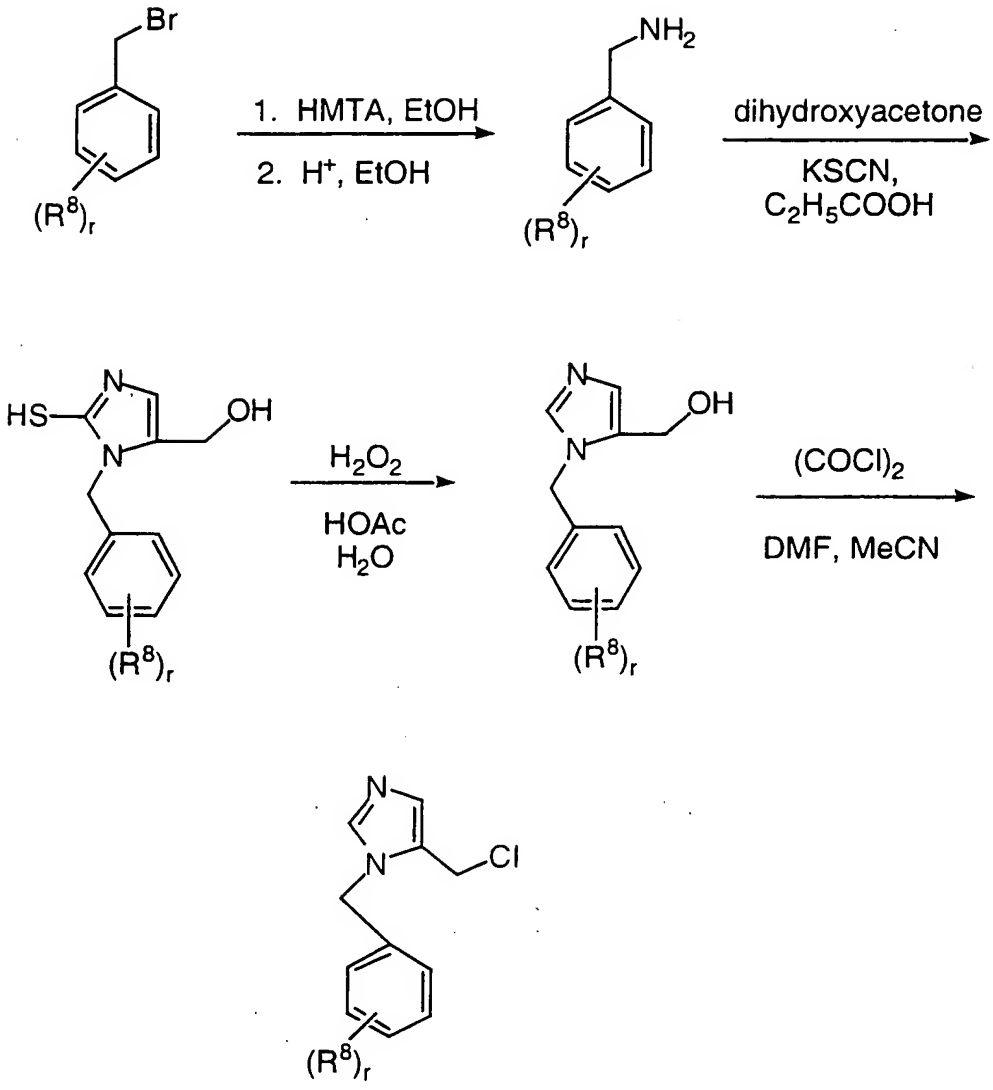
SCHEME 11


1. LDA, CO2
2. (CH3)3SiCHN2
CO2CH3
Br
N
(R8)r
Br
Zn, NiCl2(Ph3P)2
CO2CH3
excess NaBH4
CH2OH
SO3·Py, Et3N
DMSO
CHO

SCHEME 12

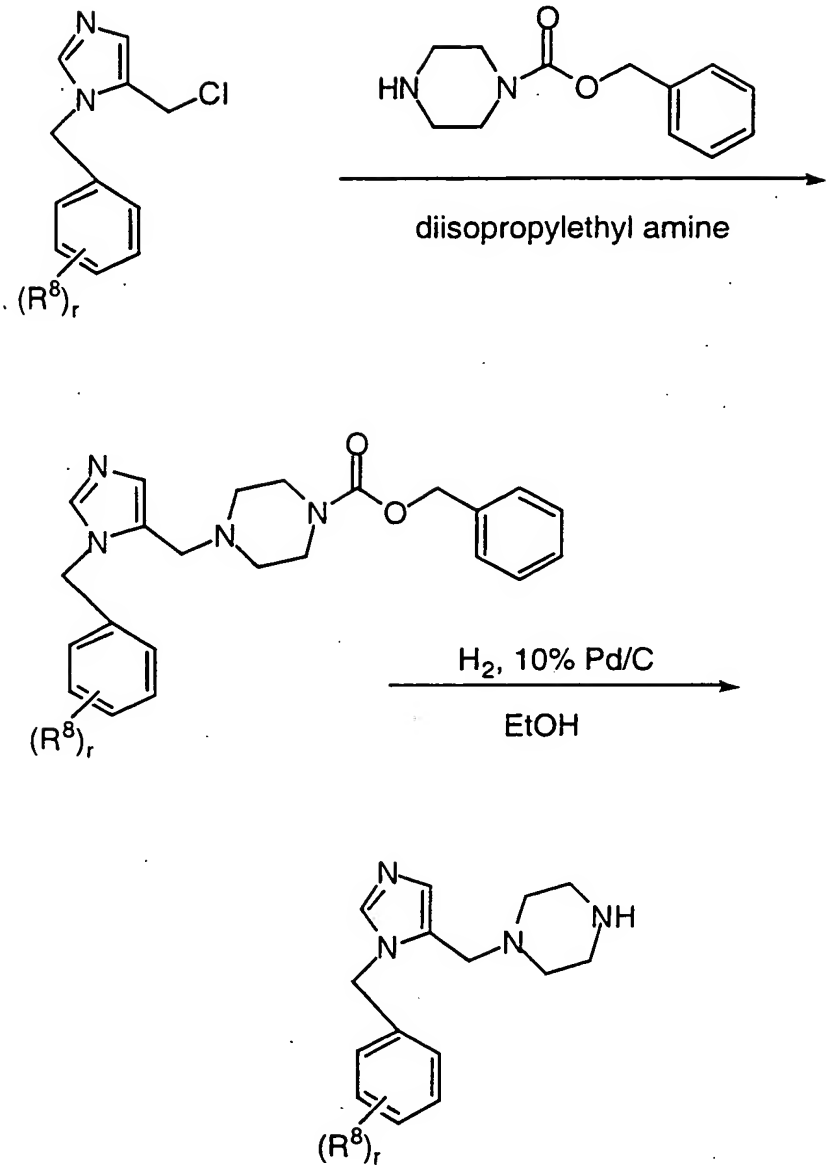

Br
N
1. LDA, CO_2
2. MeOH, H^+
CO_2CH_3
Br
N
$(R^8)_r$
MgCl
$ZnCl_2$, $NiCl_2(Ph_3P)_2$
$(R^8)_r$
CO_2CH_3
N
$NaBH_4$ (excess)
$(R^8)_r$
CH_2OH
N
SO_3·Py, Et_3N
DMSO
$(R^8)_r$
CHO
N

SCHEME 13


Br
1. HMTA, EtOH
2. H+, EtOH
NH2
dihydroxyacetone
KSCN,
C2H5COOH
HS
OH
H2O2
HOAc
H2O
(COCl)2
DMF, MeCN
Cl
(R8)r

5

SCHEME 14


N
N
Cl
(R8)r
HN
N
O
O
diisopropylethyl amine
N
N
N
O
O
(R8)r
H2, 10% Pd/C
EtOH
N
N
NH
N
(R8)r

The instant compounds are useful as pharmaceutical agents for mammals, especially for humans. These compounds may be administered to patients for use in the treatment of cancer. Examples of the type of cancer which may be treated with the compounds of this invention include, but are not limited to, colorectal carcinoma, exocrine pancreatic carcinoma, myeloid leukemias and neurological tumors. Such tumors may arise by mutations in the ras genes themselves, mutations in the proteins that can regulate Ras activity (i.e., neurofibromin (NF-1), neu, src, abl, lck, fyn) or by other mechanisms.

The compounds of the instant invention inhibit prenyl-protein transferase and the prenylation of the oncogene protein Ras. The instant compounds may also inhibit tumor angiogenesis, thereby affecting the growth of tumors (J. Rak et al. Cancer Research, 55:4575-4580 (1995)). Such anti-angiogenesis properties of the instant compounds may also be useful in the treatment of certain forms of vision deficit related to retinal vascularization.

The compounds of this invention are also useful for inhibiting other proliferative diseases, both benign and malignant, wherein Ras proteins are aberrantly activated as a result of oncogenic mutation in other genes (i.e., the Ras gene itself is not activated by mutation to an oncogenic form) with said inhibition being accomplished by the administration of an effective amount of the compounds of the invention to a mammal in need of such treatment. For example, a component of NF-1 is a benign proliferative disorder.

The instant compounds may also be useful in the treatment of certain viral infections, in particular in the treatment of hepatitis delta and related viruses (J.S. Glenn et al. Science, 256:1331-1333 (1992).

The compounds of the instant invention are also useful in the prevention of restenosis after percutaneous transluminal coronary angioplasty by inhibiting neointimal formation (C. Indolfi et al. Nature medicine, 1:541-545(1995).

The instant compounds may also be useful in the treatment and prevention of polycystic kidney disease (D.L. Schaffner et al. American Journal of Pathology, 142:1051-1060 (1993) and B. Cowley, Jr. et al.FASEB Journal, 2:A3160 (1988)).

The instant compounds may also be useful for the treatment of fungal infections.

The instant compounds may also be useful as inhibitors of proliferation of vascular smooth muscle cells and therefore useful in the prevention and therapy of arteriosclerosis and diabetic vascular pathologies.

The compounds of this invention may be administered to mammals, preferably humans, either alone or, preferably, in combination with pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The compounds can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, microcrystalline cellulose, sodium crosscarmellose, corn starch, or alginic acid; binding agents, for example starch, gelatin, polyvinyl-pyrrolidone or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to mask the unpleasant taste of the drug or delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a water soluble taste masking material such as hydroxypropylmethyl-cellulose or hydroxypropylcellulose, or a time delay material such as ethyl cellulose, cellulose acetate buryrate may be employed.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water soluble carrier such as

polyethyleneglycol or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as butylated hydroxyanisol or alpha-tocopherol.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring

phosphatides, for example soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavoring agents, preservatives and antioxidants.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, flavoring and coloring agents and antioxidant.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous solutions. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution.

The sterile injectable preparation may also be a sterile injectable oil-in-water microemulsion where the active ingredient is dissolved in the oily phase. For example, the active ingredient may be first dissolved in a mixture of soybean oil and lecithin. The oil solution then introduced into a water and glycerol mixture and processed to form a microemulation.

The injectable solutions or microemulsions may be introduced into a patient's blood-stream by local bolus injection. Alternatively, it may be advantageous to administer the solution or microemulsion in such a way as to maintain a constant circulating concentration of the instant compound. In order to maintain such a constant concentration, a continuous intravenous delivery device may be utilized. An example of such a device is the Deltec CADD-PLUS™ model 5400 intravenous pump.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension for intramuscular and subcutaneous administration. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of Formula A may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be

prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter, glycerinated gelatin, hydrogenated vegetable oils, mixtures of polyethylene glycols of various molecular weights and fatty acid esters of polyethylene glycol.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compound of Formula A are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

The compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles and delivery devices, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in the art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specific amounts, as well as any product which results, directly or indirectly, from combination of the specific ingredients in the specified amounts.

When a compound according to this invention is administered into a human subject, the daily dosage will normally be determined by the prescribing physician with the dosage generally varying according to the age, weight, sex and response of the individual patient, as well as the severity of the patient's symptoms.

In one exemplary application, a suitable amount of compound is administered to a mammal undergoing treatment for cancer. Administration occurs in an amount between about 0.1 mg/kg of body weight to about 60 mg/kg of body weight per day, preferably of between 0.5 mg/kg of body weight to about 40 mg/kg of body weight per day.

The compounds of the instant invention may also be co-administered with other well known therapeutic agents that are selected for their particular usefulness against the condition that is being treated. For example, the compounds of the instant invention may also be co-administered with other well known cancer therapeutic agents that are selected for their particular usefulness against the condition that is being treated. Included in such combinations of therapeutic agents are combinations of the instant prenyl-protein transferase inhibitors and an antineoplastic agent. It is also understood that such a combination of

antineoplastic agent and inhibitor of prenyl-protein transferase may be used in conjunction with other methods of treating cancer and/or tumors, including radiation therapy and surgery.

Examples of an antineoplastic agent include, in general, microtubule-stabilizing agents (such as paclitaxel (also known as Taxol®), docetaxel (also known as Taxotere®), epothilone A, epothilone B, desoxyepothilone A, desoxyepothilone B or their derivatives); microtubule-disruptor agents; alkylating agents, anti-metabolites; epidophyllotoxin; an antineoplastic enzyme; a topoisomerase inhibitor; procarbazine; mitoxantrone; platinum coordination complexes; biological response modifiers and growth inhibitors; hormonal/anti-hormonal therapeutic agents and haematopoietic growth factors.

Example classes of antineoplastic agents include, for example, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the taxanes, the epothilones, discodermolide, the pteridine family of drugs, diynenes and the podophyllotoxins. Particularly useful members of those classes include, for example, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, dichloro-methotrexate, mitomycin C, porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podo-phyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosidine, vindesine, leurosine, paclitaxel and the like. Other useful antineoplastic agents include estramustine, cisplatin, carboplatin, cyclophosphamide, bleomycin, tamoxifen, ifosamide, melphalan, hexamethyl melamine, thiotepa, cytarabin, idatrexate, trimetrexate, dacarbazine, L-asparaginase, camptothecin, CPT-11, topotecan, ara-C, bicalutamide, flutamide, leuprolide, pyridobenzoindole derivatives, interferons and interleukins.

The preferred class of antineoplastic agents is the taxanes and the preferred antineoplastic agent is paclitaxel.

Radiation therapy, including x-rays or gamma rays which are delivered from either an externally applied beam or by implantation of tiny radioactive sources, may also be used in combination with the instant inhibitor of prenyl-protein transferase alone to treat cancer.

Additionally, compounds of the instant invention may also be useful as radiation sensitizers, as described in WO 97/38697, published on October 23, 1997, and herein incorporated by reference.

The instant compounds may also be useful in combination with other inhibitors of parts of the signaling pathway that links cell surface growth factor receptors to nuclear signals initiating cellular proliferation. Thus, the instant compounds may be utilized in combination with a compound which has Raf antagonist activity. The instant compounds may also be co-administered with compounds that are selective inhibitors of farnesyl-protein transferase and/or compounds that are dual inhibitors of farnesyl-protein transferase and geranylgeranylprotein transferase type I. Such a selective inhibitor or dual inhibitor may be an inhibitor that is competitive with the binding of the CAAX-containing protein substrate of farnesyl-protein transferase or may be farnesyl pyrophosphate competitive inhibitors.

In particular, the compounds disclosed in the following patents and publications may be useful as farnesyl pyrophosphate-competitive inhibitor component of the instant composition: U.S. Ser. Nos. 08/254,228 and 08/435,047. Those patents and publications are incorporated herein by reference.

In practicing methods of this invention, which comprise administering, simultaneously or sequentially or in any order, two or more of a protein substrate-competitive inhibitor and a prenyl pyrophosphate-competitive inhibitor, such administration can be orally or parenterally, including intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration. It is preferred that such administration be orally. It is more preferred that such administration be orally and simultaneously. When the protein substrate-competitive inhibitor and a prenyl pyrophosphate-competitive inhibitor are administered sequentially, the administration of each can be by the same method or by different methods.

The instant compounds may also be useful in combination with an integrin antagonist for the treatment of cancer, as described in U.S. Ser. No. 09/055,487, filed April 6, 1998, which is incorporated herein by reference.

As used herein the term an integrin antagonist refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to an integrin(s) that is involved in the regulation of angiogenisis, or in the growth and invasiveness of tumor cells. In particular, the term refers to compounds which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 3$ integrin, which selectively antagonize, inhibit or counteract binding of a physiological ligand to the $\alpha v\beta 5$ integrin, which antagonize, inhibit or counteract

binding of a physiological ligand to both the αvβ3 integrin and the αvβ5 integrin, or which antagonize, inhibit or counteract the activity of the particular integrin(s) expressed on capillary endothelial cells. The term also refers to antagonists of the αvβ6, αvβ8, α1β1, α2β1, α5β1, α6β1 and α6β4 integrins. The term also refers to antagonists of any combination of αvβ3, αvβ5, αvβ6, αvβ8, α1β1, α2β1, α5β1, α6β1 and α6β4 integrins. The instant compounds may also be useful with other agents that inhibit angiogenisis and thereby inhibit the growth and invasiveness of tumor cells, including, but not limited to angiostatin and endostatin.

Similarly, the instant compounds may be useful in combination with agents that are effective in the treatment and prevention of NF-1, restenosis, polycystic kidney disease, infections of hepatitis delta and related viruses and fungal infections.

If formulated as a fixed dose, such combination products employ the combinations of this invention within the dosage range described below and the other pharmaceutically active agent(s) within its approved dosage range. Combinations of the instant invention may alternatively be used sequentially with known pharmaceutically acceptable agent(s) when a multiple combination formulation is inappropriate.

EXAMPLES

Examples provided are intended to assist in a further understanding of the invention. Particular materials employed, species and conditions are intended to be further illustrative of the invention and not limitative of the reasonable scope thereof.

EXAMPLE 1

Preparation of 1-(4-cyanobenzyl)-5-chloromethyl imidazole HCl salt

Step 1: Preparation of 4-Cyanobenzylamine

Method 1 (Hydrochloride salt): A 72 liter vessel was charged with 190 proof ethanol (14.4 L) followed by the addition of 4-cyanobenzylbromide (2.98 kg) and HMTA (2.18 kg) at ambient temperature. The mixture was heated to about 72-

75°C over about 60 min. On warming, the solution thickens and additional ethanol (1.0 liter) was added to facilitate stirring. The batch was aged at about 72-75°C for about 30 min.

The mixture was allowed to cool to about 20°C over about 60 min, and HCl gas (2.20 kg) was sparged into the slurry over about 4 hours during which time the temperature rose to about 65°C. The mixture was heated to about 70-72°C and aged for about 1 hour. The slurry was cooled to about 30°C and ethyl acetate (22.3 L) added over about 30 min. The slurry was cooled to about -5°C over about 40 min and aged at about -3 to about -5°C for about 30 min. The mixture was filtered and the crystalline solid was washed with chilled ethyl acetate (3 x 3 L). The solid was dried under a N_2 stream for about 1 hour before charging to a 50 liter vessel containing water (5.5 L). The pH was adjusted to about 10-10.5 with 50% NaOH (4.0 kg) maintaining the internal temperature below about 30°C. At about 25°C, methylene chloride (2.8 L) was added and stirring continued for about 15 min. The layers were allowed to settle and the lower organic layer was removed. The aqueous layer was extracted with methylene chloride (2 x 2.2 L). The combined organic layers were dried over potassium carbonate (650 g). The carbonate was removed via filtration and the filtrate concentrated in vacuo at about 25°C to give a free base as a yellow oil.

The oil was transferred to a 50 liter vessel with the aid of ethanol (1.8 L). Ethyl acetate (4.1 L) was added at about 25°C. The solution was cooled to about 15°C and HCl gas (600 g) was sparged in over about 3 hours, while keeping batch temperature below about 40°C. At about 20-25 °C, ethyl acetate (5.8 L) was added to the slurry, followed by cooling to about -5°C over about 1 hour. The slurry was aged at about -5°C for about 1 hour and the solids isolated via filtration. The cake was washed with a chilled mixture of EtOAc/EtOH (9:1 v/v) (1 x 3.8 L), then the cake was washed with chilled EtOAc (2 x 3.8 L). The solids were dried in vacuo at about 25°C to provide the above-titled compound.

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.83-7.79 (d, 2H), 7.60-7.57 (d, 2H), 4.79 (s, 2H), 4.25 (s, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 149.9, 139.8, 134.2, 131.2, 119.7, 113.4, 49.9, 49.5, 49.2, 48.8, 48.5, 48.2, 43.8.

Method 2 (phosphate salt): A slurry of HMTA in 2.5 L EtOH was added gradually over about 30 min to about 60 min to a stirred slurry of cyanobenzyl-

bromide in 3.5 L EtOH and maintained at about 48-53°C with heating & cooling in a 22L neck flask (small exotherm). Then the transfer of HMTA to the reaction mixture was completed with the use of 1.0 L EtOH. The reaction mixture was heated to about 68-73°C and aged at about 68-73°C for about 90 min. The reaction mixture was a slurry containing a granular precipitate which quickly settled when stirring stopped.

The mixture was cooled to a temperature of about 50°C to about 55°C. Propionic acid was added to the mixture and the mixture was heated and maintained at a temperature of about 50°C to about 55°C. Phosphoric acid was gradually added over about 5 min to about 10 min, maintaining the reaction mixture below about 65 °C to form a precipitate-containing mixture. Then the mixture was gradually warmed to about 65°C to about 70°C over about 30 min and aged at about 65°C to about 70°C for about 30 min. The mixture was then gradually cooled to about 20-25°C over about 1 hour and aged at about 20-25°C for about 1 hour.

The reaction slurry was then filtered. The filter cake was washed four times with EtOH, using the following sequence, 2.5 L each time. The filter cake was then washed with water five times, using 300 mL each time. Finally, the filter cake was washed twice with MeCN (1.0 L each time) and the above identified compound was obtained.

Step 2: Preparation of 1-(4-Cyanobenzyl)-2-Mercapto-5-Hydroxymethylimidazole

7% water in acetonitrile (50 mL) was added to a 250 mL roundbottom flask. Next, an amine phosphate salt (12.49 g), prepared as described in Step 1, was added to the flask. Next potassium thiocyanate (6.04 g) and dihydroxyacetone (5.61 g) was added. Lastly, propionic acid (10.0 mL) was added. Acetonitrile/water 93:7 (25 mL) was used to rinse down the sides of the flask. This mixture was then heated to 60°C, aged for about 30 minutes and seeded with 1% thioimidazole. The mixture was then aged for about 1.5 to about 2 hours at 60°C. Next, the mixture was heated to 70°C, and aged for 2 hours. The temperature of the mixture was then cooled to room temperature and was aged overnight. The thioimidazole product was obtained by vacuum filtration. The filter cake was washed four times acetonitrile (25 mL each time) until the filtrates became nearly colorless. Then the filter cake was washed three times with water (approximately 25-50 mL each time) and dried in vacuo to obtain the above-identified compound.

Step 3: Preparation of 1-(4-Cyanobenzyl)-5-Hydroxymethylimidazole

A 1L flask with cooling/heating jacket and glass stirrer (Lab-Max) was charged with water (200 mL) at 25°C. The thioimidazole (90.27 g), prepared as described in Step 2, was added, followed by acetic acid (120 mL) and water (50 mL) to form a pale pink slurry. The reaction was warmed to 40°C over 10 minutes. Hydrogen peroxide (90.0 g) was added slowly over 2 hours by automatic pump maintaining a temperature of 35-45°C. The temperature was lowered to 25°C and the solution aged for 1 hour.

The solution was cooled to 20°C and quenched by slowly adding 20% aqueous Na_2SO_3 (25 mL) maintaining the temperature at less than 25°C. The solution was filtered through a bed of DARCO G-60 (9.0 g) over a bed of SolkaFlok (1.9 g) in a sintered glass funnel. The bed was washed with 25 mL of 10% acetic acid in water.

The combined filtrates were cooled to 15°C and a 25% aqueous ammonia was added over a 30 minute period, maintaining the temperature below 25°C, to a pH of 9.3. The yellowish slurry was aged overnight at 23°C (room temperature). The solids were isolated via vacuum filtration. The cake (100 mL wet volume) was washed with 2 x 250 mL 5% ammonia (25%) in water, followed by 100 mL of ethyl acetate. The wet cake was dried with vacuum/N_2 flow and the above-titled compound was obtained.

^1H NMR (250 MHz, $CDCl_3$): δ 7.84-7.72 (d, 2H), 7.31-7.28 (d, 2H), 6.85 (s, 1H), 5.34 (s, 2H), 5.14-5.11 (t, 1H), 4.30-4.28 (d, 2H), 3.35 (s, 1H).

Step 4: Preparation of 1-(4-cyanobenzyl)-5-chloromethyl imidazole HCl salt

Method 1: 1-(4-Cyanobenzyl)-5-hydroxymethylimidazole (1.0 kg), prepared as described above in Step 3, was slurried with DMF (4.8 L) at 22°C and then cooled to -5°C. Thionyl chloride (390 mL) was added dropwise over 60 min during which time the reaction temperature rose to a maximum of 9°C. The solution became nearly homogeneous before the product began to precipitate from solution. The slurry was warmed to 26°C and aged for 1 h.

The slurry was then cooled to 5°C and 2-propanol (120 mL) was added dropwise, followed by the addition of ethyl acetate (4.8 L). The slurry was aged at 5°C for 1 h before the solids were isolated and washed with chilled ethyl acetate (3 x

1 L). The product was dried in vacuo at 40°C overnight to provide the above-titled compound.

^{1}H NMR (250 MHz DMSO-d6): δ 9.44 (s, 1H), 7.89 (d, 2H, 8.3 Hz), 7.89 (s, 1H), 7.55 (d, 2H, 8.3 Hz), 5.70 (s, 2H), 4.93 (s, 2H). ^{13}C NMR (75.5 MHz DMSO-d6): δ_c 139.7, 137.7, 132.7, 130.1, 128.8, 120.7, 118.4, 111.2, 48.9, 33.1.

Method 2: To an ice cold solution of dry acetonitrile (3.2 L, 15 L/Kg hydroxymethylimidazole) was added 99% oxalyl chloride (101 mL, 1.15 mol, 1.15 equiv.), followed by dry DMF (178 mL, 2.30 mol, 2.30 equiv.), at which time vigorous evolution of gas was observed. After stirring for about 5 to 10 min following the addition of DMF, solid hydroxymethylimidazole (213 g, 1.00 mol), prepared as described above in Step 3, was added gradually. After the addition, the internal temperature was allowed to warm to a temperature of about 23°C to about 25°C and stirred for about 1 to 3 hours. The mixture was filtered, then washed with dry acetonitrile (400 mL displacement wash, 550 mL slurry wash, and a 400 mL displacement wash). The solid was maintained under a N_2 atmosphere during the filtration and washing to prevent hydrolysis of the chloride by adventitious H_2O. This yielded the crystalline form of the chloromethylimidazole hydrochloride.

^{1}H NMR (250 MHz DMSO-d6): δ 9.44 (s, 1H), 7.89 (d, 2H, 8.3 Hz), 7.89 (s, 1H), 7.55 (d, 2H, 8.3 Hz), 5.70 (s, 2H), 4.93 (s, 2H). ^{13}C NMR (75.5 MHz DMSO-d6): δ_c 139.7, 137.7, 132.7, 130.1, 128.8, 120.7, 118.4, 111.2, 48.9, 33.1.

Method 3: To an ice cold solution of dry DMF (178 mL, 2.30 mol, 2.30 equiv.) in dry acetonitrile (2.56 L, 12 L/Kg Hydroxymethylimidazole) was added oxalyl chloride (101 mL, 1.15 mol, 1.15 equiv). The heterogeneous mixture in the reagent vessel was then transferred to a mixture of hydroxymethylimidazole (213 g, 1.00 mol), prepared as described in Step 3 above, in dry acetonitrile (1.7 L, 8 L/Kg hydroxymethylimidazole). Additional dry acetonitrile (1.1 - 2.3 L, 5 - 11 L/Kg hydroxymethylimidazole) was added to the remaining solid Vilsmeier reagent in the reagent vessel. This, now nearly homogenous, solution was transferred to the reaction vessel at $T_i \leq +6$ C. The reaction vessel temperature was warmed to a temperature of about 23°C to about 25°C and stirred for about 1 to 3 hours. The mixture was then

cooled to 0°C and aged 1 h. The solid was filtered and washed with dry, ice cold acetonitrile (400 mL displacement wash, 550 mL slurry wash, and a 400 mL displacement wash). The solid was maintained under a N_2 atmosphere during the filtration and washing to prevent hydrolysis of the chloride by adventitious H_2O. This yielded the crystalline form of the chloromethylimidazole hydrochloride.

EXAMPLE 2

Preparation Of 1-(4'-Cyanobenzyl) imidazol-5-ylmethyl piperazine

Step 1: Preparation of 1-(4'-Cyanobenzyl) imidazol-5-ylmethyl piperazine-4-carboxylic acid benzyl ester

To an acetonitrile solution of 1-(4'-cyanobenzyl)-5-chloromethylimidazole (7.45 mmol), prepared as described in Example 1, Step 4, and diisopropylethylamine (22.4 mmol) was added 1-benzyl 1-piperazine carboxylate (10.4mmol). This solution was stirred for 4.0 hours at 80°C. The product was isolated after silica column purification.

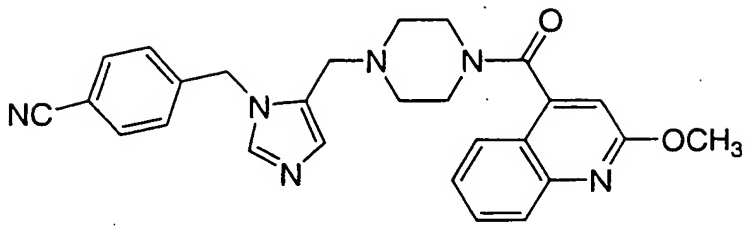
^{1}H-NMR ($CDCl_3$): δ 7.65 (d, 2H); 7.55 (s, 1H); 7.38 (m, 5H); 7.15 (d, 2H); 7.0 (s, 1H); 5.3 (s, 2H); 5.1 (s, 1H); 3.4 (m, 4H); 3.3 (s, 2H); 2.3 (m, 4H).

Step 2: Preparation of 1-(4'-Cyanobenzyl) imidazol-5-ylmethyl piperazine

The product from Step 1 (6.17 mmol) was dissolved in absolute ethanol followed by the introduction of 10% Pd/C catalyst then hydrogen under atmospheric pressure. The catalyst was removed via filtration through filter-aid and the product was isolated by removing the solvent under reduced pressure.

^{1}H-NMR (CD_3OD): δ 7.8 (s, 1H); 7.75 (d, 2H); 7.3 (d, 2H); 6.9 (s, 1H); 5.45 (s, 2H); 3.3 (m, 4H); 2.6 (s, 2H); 2.3 (m, 4H).

EXAMPLE 3



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-methoxyquinolin-4-oyl)piperazine trihydrochloride

Step 1: Preparation of 2-methoxyquinoline-4-carboxylic acid methyl ester

To a solution of 2-hydroxyquinoline-4-carboxylic acid (125 mg, 0.661 mmol) and silver carbonate (456 mg, 1.65 mmol) in chloroform (10 mL) was added methyl iodide (411 μL, 6.61 mmol). The reaction mixture was stirred for 48 hours, filtered, and partitioned between methylene chloride (10 mL) and water (10 mL). The layers were separated and the aqueous layer was extracted with methylene chloride (2 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the title compound as a yellow oil.

Step 2: Preparation of 2-methoxyquinoline-4-carboxylic acid

A solution of the ester from Step 1 (144 mg, 0.661 mmol) in THF (3 mL)/water (1 mL) and lithium hydroxide hydrate (41.7 mg, 0.994 mmol) was stirred for 2.5 hours, poured onto 10% HCl, and extracted with ethyl acetate (2 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the title product as a white solid.

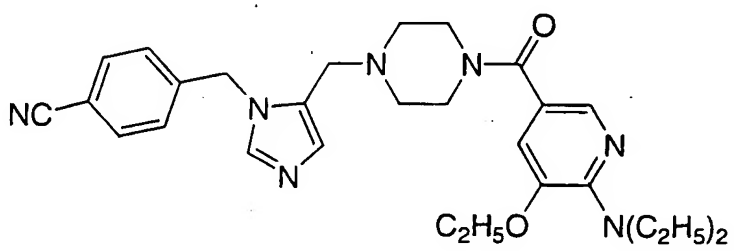
Step 3: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-methoxyquinolin-4-oyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 21.7 mg, 0.0556 mmol), the acid from Step 2 (13.6 mg, 0.0667 mmol), EDC hydrochloride (11.7 mg, 0.0612 mmol), HOBT (8.27 mg, 0.0612 mmol), and N,N-diisopropylethylamine (48.4 μL, 0.278 mmol) were stirred in dry, degassed DMF (500 μL) at 20°C under nitrogen. The reaction was stirred overnight and then

injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 467. Anal. Calc. for $C_{27}H_{26}N_6O_2 \cdot 3.0\ HCl \cdot 1.10\ H_2O$: C, 54.43; H, 5.28; N, 14.11. Found: C, 54.49; H, 5.43; N, 13.71.

EXAMPLE 4



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-diethylamino-3-ethoxypyrid-5-oyl)piperazine trihydrochloride

Step 1: Preparation of 6-chloro-5-hydroxy nicotinic acid methyl ester

A solution of 5-hydroxy nicotinic acid methyl ester(1.00 g, 6.53 mmol) and N-chlorosuccinimide (1.74 g, 13.1 mmol) in DMF (20 mL) was heated at 90°C for 20 hours. The solvent was removed *in vacuo* and the residue partitioned between methylene chloride (50 mL) and 10% HCl (50 mL). The layers were separated and the aqueous layer was extracted with methylene chloride (3 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The titled product was isolated as a brown solid after recrystallization from methylene chloride.

Step 2: Preparation of 6-chloro-5-ethoxy nicotinic acid

To a solution of product from Step 1 (188 mg, 1.00 mmol) and potassium hydroxide (112 mg, 2.00 mmol) in DMSO (2 mL) was added ethyl iodide (71.5 µL, 1.20 mmol). The solution was stirred for 16 hours, poured onto 10% HCl (10 mL), and extracted with methylene chloride (3 x 10 mL). The combined organic

layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the titled product as a white solid.

Step 3: Preparation of 6-diethylamino-5-ethoxy nicotinic acid

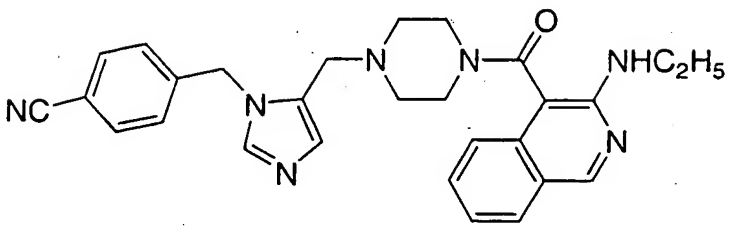
A solution of the product from Step 2 (100 mg, 0.496 mmol) in diethylamine (2.5 mL)/ ethanol (2.5 mL) was heated in a sealed pressure tube at 145°C for 72 hours. The rection slurry was filtered and concentrated *in vacuo*. The crude product was purified on a C18 preparative HPLC column using a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min to provide the titled product as a white solid.

Step 4: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-diethylamino-3-ethoxypyrid-5-oyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 21.6 mg, 0.0554 mmol), the acid from Step 2 (15.0 mg, 0.0426 mmol), EDC hydrochloride (12.2 mg, 0.0639 mmol), HOBT (8.63 mg, 0.0639 mmol), and N,N-diisopropylethylamine (37.1 µL, 0.213 mmol) were stirred in dry, degassed DMF (500 µL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 502. Anal. Calc. for $C_{28}H_{35}N_7O_2 \cdot 4.4\ HCl \cdot 0.80\ EtOAc$: C, 51.15; H, 6.30; N, 13.39. Found: C, 51.12; H, 6.08; N, 13.37.

EXAMPLE 5



L-452,958

Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-ethylamino-4-isoquinolinoyl)piperazine trihydrochloride

Step 1: Preparation of 3-(ethylamino)isoquinoline-4-carboxylic acid ethyl ester

To a solution of 3-aminoisoquinoline-4-carboxylic acid ethyl ester (166 mg, 0.768 mmol), prepared by the method of Suzuki *et al* (*Synthesis*, 1995, 763), in THF (2 mL)/DMPU (1 mL) at 0°C was added lithium bis(trimethylsilyl)amide (1.0M in THF, 2.30 mL). The solution was stirred for 1 hour and then ethyl iodide (137 µL, 2.30 mmol) was added. The solution was stirred for 16 hours, poured onto brine (20 mL), and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified on a C18 preparative HPLC column using a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min to yield the titled product as a white solid.

Step 2: Preparation of 3-(ethylamino)isoquinoline-4-carboxylic acid hydrochloride

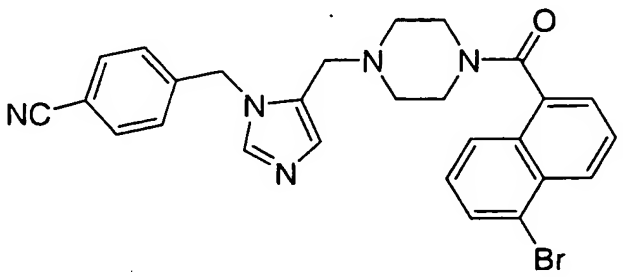
To a solution of product from Step 1 (49.5 mg, 0.203 mmol) in ethanol (3 mL) was added sodium hydroxide (16.2 mg, 0.405 mmol). The solution was heated at reflux for 2 hours, quenched by the addition of HCl (1M in ether, 1 mL), and concentrated *in vacuo* to yield the titled product as a yellow solid.

Step 3: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-ethylamino-4-isoquinolinoyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 51.0 mg, 0.131 mmol), the acid from Step 2 (49.5 mg, 0.196 mmol), EDC hydrochloride (27.5 mg, 0.144 mmol), HOBT (19.4 mg, 0.144 mmol), and N,N-diisopropylethylamine (114 µL, 0.653 mmol) were stirred in dry, degassed DMF (1 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 480. Anal. Calc. for $C_{28}H_{29}N_7O_1 \cdot 5.1\ HCl \cdot 0.30\ Et_2O$: C, 50.99; H, 5.44; N, 14.26. Found: C, 51.04; H, 5.47; N, 14.28.

EXAMPLE 6

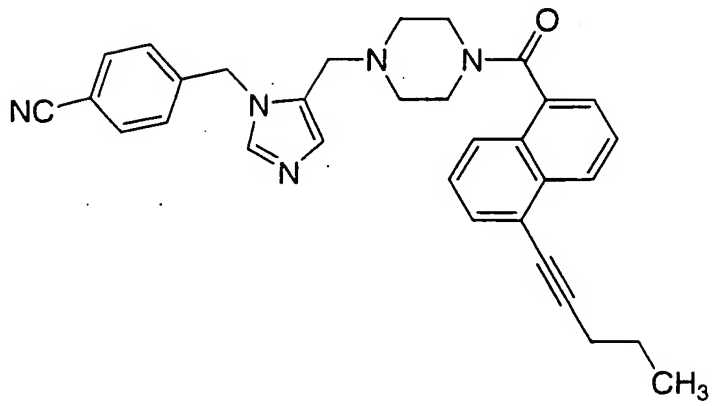


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-bromo-1-naphthoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 190 mg, 0.486 mmol), 5-bromo-1-napthoic acid (122 mg, 0.486 mmol, prepared as described in *Journal of the Chemical Society*, 1927, 3098), EDC hydrochloride (102 mg, 0.535 mmol), HOBT (72.2 mg, 0.535 mmol), and N,N-diisopropylethylamine (423 µL, 2.43 mmol) were stirred in dry, degassed DMF (2 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 515. Anal. Calc. for $C_{27}H_{24}Br_1N_5O_1 \cdot 2.2\ HCl$: C, 54.70; H, 4.45; N, 11.81. Found: C, 54.71; H, 4.69; N, 11.42.

EXAMPLE 7

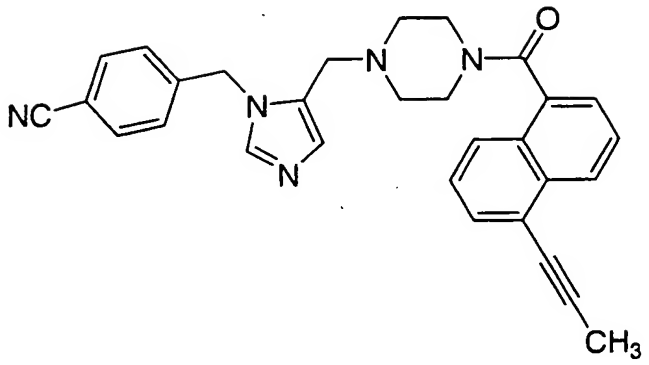


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(pent-1-ynyl)-1-naphthoyl]piperazine bishydrochloride

Bromide from Example 6 (30.8 mg, 0.0524 mmol), 1-pentyne (20.7 μL, 0.210 mmol), dichlorobis(triphenylphosphine)palladium (3.68 mg, 0.00524 mmol), copper (I) iodide (2.00 mg, 0.0149 mmol), and triethylamine (36.5 μL, 0.262 mmol) were heated in dry, degassed DMF (0.5 mL) at 100°C in a sealed tube. The reaction was heated overnight, filtered, and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 502. Anal. Calc. for $C_{32}H_{31}N_5O_1 \cdot 2.3\ HCl \cdot 2.5\ THF$: C, 65.87; H, 7.02; N, 9.15. Found: C, 65.81; H, 6.71; N, 8.68.

EXAMPLE 8

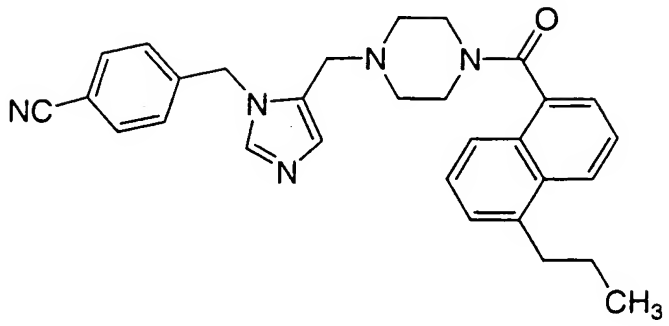


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(prop-1-ynyl)-1-naphthoyl]piperazine bishydrochloride

Bromide from Example 6 (33.9 mg, 0.0577 mmol), tributyl(propynyl)tin (69.7 µL, 0.231 mmol), potassium carbonate (39.9 mg, 0.289 mmol), and tetrakis(triphenylphosphine)palladium (6.67 mg, 0.00577 mmol) were heated in dry, degassed DMF (0.5 mL) at 100°C in a sealed tube. The reaction was heated overnight, filtered, and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 474. Anal. Calc. for $C_{30}H_{27}N_5O_1 \cdot 3.0\ HCl \cdot 0.55\ Et_2O$: C, 62.18; H, 5.75; N, 11.26. Found: C, 62.13; H, 5.56; N, 11.24.

EXAMPLE 9

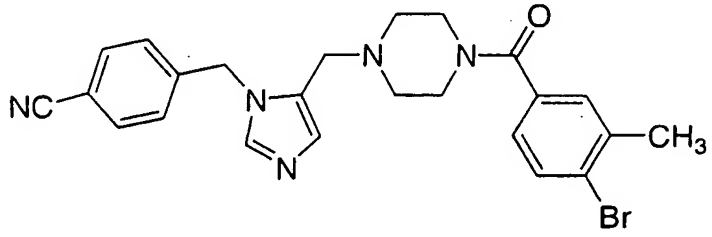


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-propyl-1-naphthoyl)piperazine bishydrochloride

Product from Example 8 (10.0 mg, 0.0211 mmol), and 10% palladium on carbon (10 mg) were suspended in methanol (2 mL) and placed under a hydrogen atmosphere for 1.5 hours. The reaction solution was filtered and concentrated *in vacuo*. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 478. Anal. Calc. for $C_{30}H_{31}N_5O_1 \cdot 2.0\ HCl \cdot 0.75\ CH_2Cl_2$: C, 60.12; H, 5.66; N, 11.40. Found: C, 60.16; H, 5.45; N, 11.31.

EXAMPLE 10



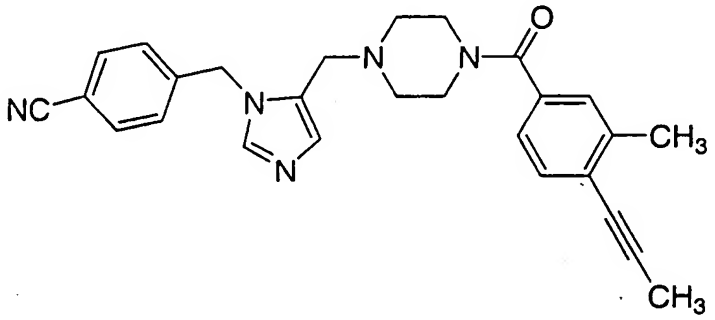
Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-bromo-3-methylbenzoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 172 mg, 0.441 mmol), 4-bromo-3-methylbenzoic acid (94.8 mg, 0.441 mmol), EDC hydrochloride (92.9 mg, 0.485 mmol), HOBT (65.5 mg, 0.485 mmol), and N,N-diisopropylethylamine (384 µL, 2.20 mmol) were stirred in dry,

degassed DMF (1 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 478. Anal. Calc. for $C_{24}H_{24}Br_1N_5O_1 \cdot 2.4\ HCl \cdot 0.45\ Et_2O$: C, 51.86; H, 5.21; N, 11.72. Found: C, 51.82; H, 5.32; N, 11.73.

EXAMPLE 11

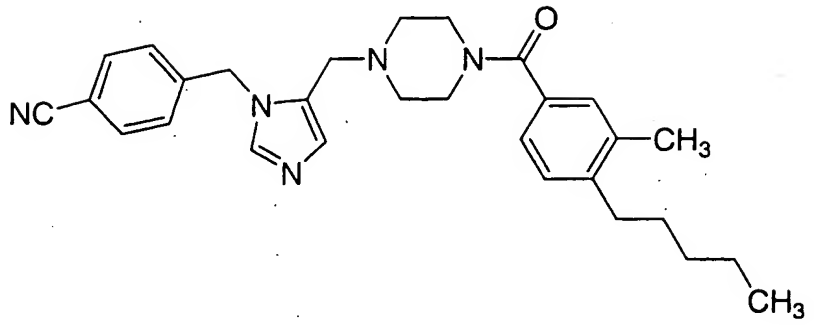


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl -4-(prop-1-ynyl)benzoyl]piperazine bishydrochloride

Bromide from Example 10 (34.0 mg, 0.0599 mmol), tributyl(propynyl)tin (78.9 mg, 0.240 mmol), potassium carbonate (41.4 mg, 0.300 mmol), and terakis(triphenylphosphine)palladium (6.67 mg, 0.00577 mmol) were heated in dry, degassed DMF (1 mL) at 100°C in a sealed tube. The reaction was heated for 2 hours, filtered, and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 438. Anal. Calc. for $C_{27}H_{27}N_5O_1 \cdot 2.1\ HCl \cdot 1.9\ EtOAc$: C, 61.02; H, 6.54; N, 10.34. Found: C, 60.96; H, 6.53; N, 10.35.

EXAMPLE 12



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methyl-4-pentylbenzoyl)piperazine bishydrochloride

Step 1: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl-4-(pent-1-ynyl)benzoyl]piperazine bishydrochloride

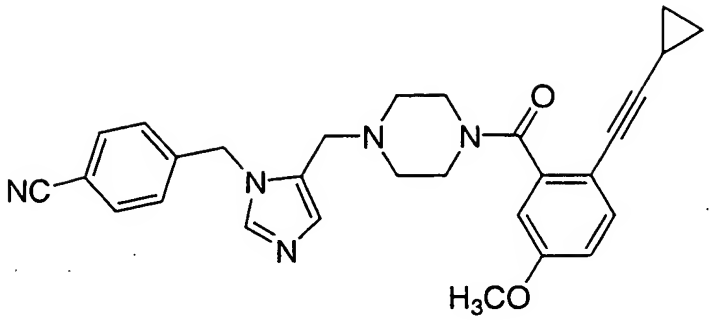
Bromide from Example 10 (34.9 mg, 0.0615 mmol), 1-pentyne (30.3 µL, 0.308 mmol), dichlorobis(triphenylphosphine)palladium (4.30 mg, 0.00615 mmol), copper (I) iodide (2.30 mg, 0.0123 mmol), and triethylamine (42.9 µL, 0.308 mmol) were heated in dry, degassed DMF (1 mL) at 100°C in a sealed tube. The reaction was heated for 48 hours, filtered, and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

Step 2: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methyl-4-pentylbenzoyl)piperazine bishydrochloride

Product from Step 1 (10.0 mg, 0.0215 mmol), and 10% palladium on carbon (10 mg) were suspended in methanol (1 mL) and placed under a hydrogen atmosphere for 4.5 hours. The reaction solution was filtered and concentrated *in vacuo*. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 470. Anal. Calc. for $C_{29}H_{35}N_5O_1 \cdot 3.1\ HCl \cdot 1.7\ EtOAc$: C, 58.73; H, 7.10; N, 9.62. Found: C, 58.77; H, 6.99; N, 9.58.

EXAMPLE 13



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-cyclopropyleth-ynyl-5-methoxybenzoyl)piperazine bishydrochloride

Step 1: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-bromo-5-methoxybenzoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 1.50 g, 3.84 mmol), 2-bromo-5-methoxybenzoic acid (887 mg, 3.84 mmol), EDC hydrochloride (810 mg, 4.22 mmol), HOBT (571 mg, 4.22 mmol), and N,N-diisopropylethylamine (3.34 mL, 19.2 mmol) were stirred in dry, degassed DMF (10 mL) at 20°C under nitrogen. The solution was stirred for 48 hours, poured onto sat. aq. $NaHCO_3$ (50 mL), and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide a yellow oil. The crude product was purified by column chromatography (5 → 10% MeOH/$CHCl_3$) and converted to the HCl salt to provide the title compound as a white solid.

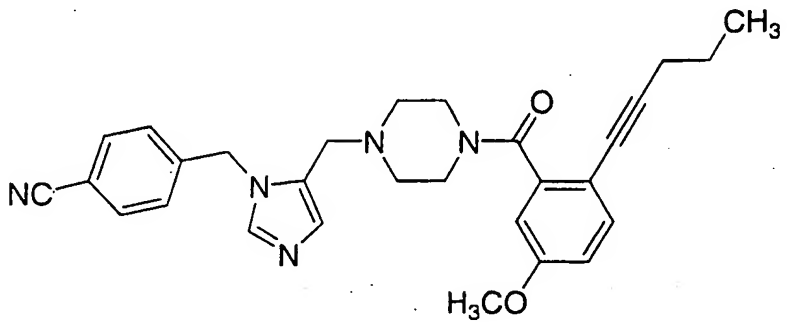
Step 2: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-cyclopropylethynyl-5-methoxybenzoyl)piperazine bishydrochloride

Bromide from Step 1 (118 mg, 0.209 mmol), tributyl-(cyclopropylethynyl)tin (148 mg, 0.417 mmol), terakis(triphenylphosphine)palladium (12.0 mg, 0.0104 mmol), and potassium carbonate (144 mg, 1.04 mmol) were heated in dry, degassed DMF (2 mL) at 100°C in a sealed tube. The reaction was heated for 2 hours, filtered, and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous

TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 480. Anal. Calc. for $C_{29}H_{29}N_5O_2 \cdot 1.5\ HCl \cdot 1.8\ CH_2Cl_2$: C, 54.08; H, 5,02; N, 10.26. Found: C, 54.08; H, 5.13; N, 10.26.

EXAMPLE 14



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-methoxy-2-pent-1-ynylbenzoyl)piperazine bishydrochloride

Step 1: Preparation of 5-methoxy-2-pent-1-ynylbenzoic acid methyl ester

5-methoxy-2-bromobenzoic acid methyl ester (528 mg, 2.15 mmol), 1-pentyne (424 μL, 4.31 mmol), triphenylphosphine (141 mg, 0.538 mmol), dichlorobis(triphenylphosphine)palladium (75.5 mg, 0.108 mmol), and copper (I) iodide (102 mg, 0.538 mmol) were heated in triethylamine (10 mL) at 100°C in a sealed tube. The reaction was heated for 24 hours, poured onto sat. aq. $NaHCO_3$ (50 mL), and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide a yellow oil. The crude product was injected on a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min to provide the title compound as a white solid..

Step 2: Preparation of 5-methoxy-2-(pent-1-ynyl)benzoic acid methyl ester

A solution of the ester from Step 1 (150 mg, 0.646 mmol) and potassiumium hydroxide (54.4 mg, 0.969 mmol) in dioxane (2 mL)/water (1 mL) was stirred for 3.5 hours, poured onto 10% HCl (10 mL), and extracted with ethyl acetate

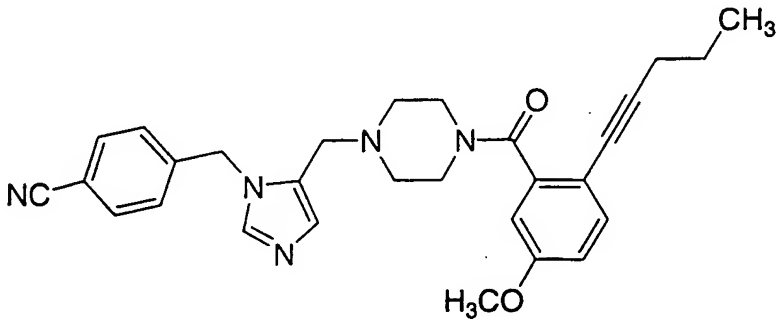
(3 x 10 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide the title product as an off-white solid which was sufficiently pure for use in the next step.

Step 3: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-methoxy-2-(pent-1-ynyl)benzoyl]piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 81.1 mg, 0.208 mmol), the acid from Step 2 (45.3 mg, 0.208 mmol), EDC hydrochloride (43.8 mg, 0.228 mmol), HOBT (30.9 mg, 0.228 mmol), and N,N-diisopropylethylamine (181 µL, 1.04 mmol) were stirred in dry, degassed DMF (2 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 482. Anal. Calc. for $C_{29}H_{31}N_5O_2 \cdot 3.8\ HCl \cdot 0.70\ EtOAc$: C, 56.16; H, 5.98; N, 10.30. Found: C, 56.19; H, 6.37; N, 10.60.

EXAMPLE 15



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethynylbenzoyl)piperazine bishydrochloride

Step 1: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-iodobenzoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 716 mg, 1.83 mmol), 5-chloro-2-iodobenzoic acid (518 mg, 1.83

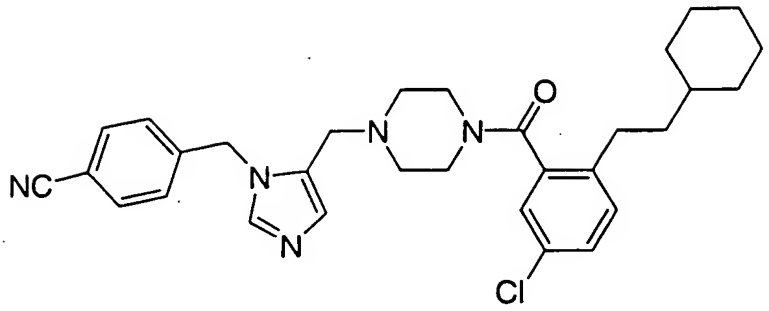
mmol), EDC hydrochloride (386 mg, 2.02 mmol), HOBT (272 mg, 2.02 mmol), and N,N-diisopropylethylamine (1.60 mL, 9.16 mmol) were stirred in dry, degassed DMF (10 mL) at 20 °C under nitrogen. The solution was stirred for 16 hours, poured onto sat. aq. $NaHCO_3$ (50 mL), and extracted with methylene chloride (3 x 50 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to provide a yellow oil. The crude product was purified by column chromatography (0 → 5% MeOH/CH_2Cl_2) and converted to the HCl salt to provide the title compound as a white solid.

Step 2: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethynylbenzoyl)piperazine bishydrochloride

Iodide from Step 1 (118 mg, 0.190 mmol), cyclohexylacetylene (51.0 µL, 0.381 mmol), dichlorobis(triphenylphosphine)palladium (13.4 mg, 0.0190 mmol), copper (I) iodide (7.20 mg, 0.0380 mmol), and triethylamine (132 µL, 0.950 mmol) were heated in DMF (3 mL) at 100°C in a sealed tube. The reaction was heated for 4 hours, filtered, injected on a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 526. Anal. Calc. for $C_{31}H_{32}Cl_1N_5O_1 \cdot 2.9\ HCl \cdot 0.50\ EtOAc$: C, 58.64; H, 5.80; N, 10.36. Found: C, 58.69; H, 5.73; N, 10.34.

EXAMPLE 16

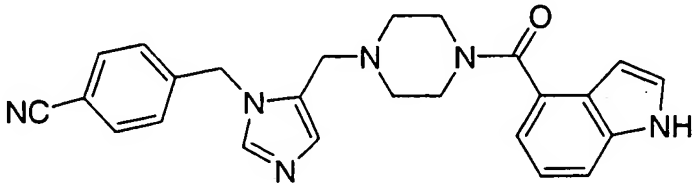


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethylbenzoyl)piperazine bishydrochloride

Product from Example 15, Step 2 (10.0 mg, 0.0190 mmol), and 10% palladium on carbon (10 mg) were suspended in methanol (2 mL) and placed under a hydrogen atmosphere for 8 hours. The reaction solution was filtered and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 530. Anal. Calc. for $C_{31}H_{36}Cl_1N_5O_1 \cdot 2.1\ HCl \cdot 1.45\ EtOAc$: C, 60.33; H, 6.83; N, 9.56. Found: C, 60.32; H, 6.89; N, 9.57.

EXAMPLE 17



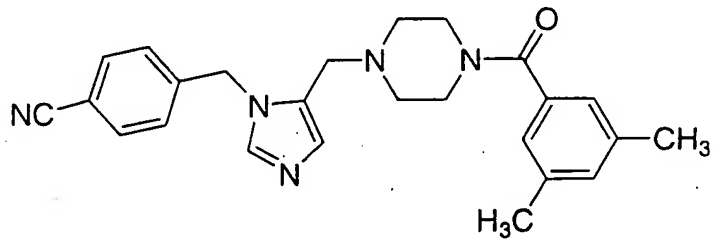
Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-indoloyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 22.0 mg, 0.0562 mmol), indole-4-carboxylic acid (9.06 mg, 0.0562 mmol), EDC hydrochloride (11.8 mg, 0.0618 mmol), HOBT (8.35 mg, 0.0618 mmol), and N,N-diisopropylethylamine (48.9 µL, 0.281 mmol) were stirred in dry,

degassed DMF (0.5 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 425. Anal. Calc. for $C_{25}H_{24}N_6O_1 \cdot 3.0\ HCl \cdot 1.15\ Et_2O$: C, 57.42; H, 6.27; N, 13.58. Found: C, 57.74; H, 6.28; N, 13.61.

EXAMPLE 18

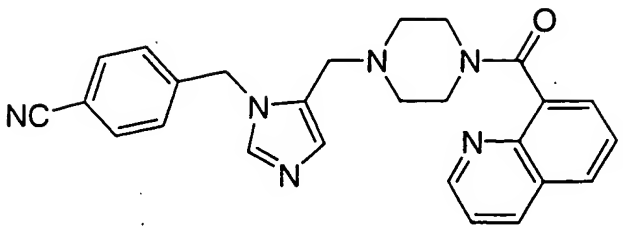


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,5-dimethylbenzoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 20.1 mg, 0.0514 mmol), 3,5-dimethylbenzoic acid (7.72 mg, 0.0514 mmol), EDC hydrochloride (10.8 mg, 0.0565 mmol), HOBT (7.64 mg, 0.0565 mmol), and N,N-diisopropylethylamine (44.8 µL, 0.257 mmol) were stirred in dry, degassed DMF (0.5 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 414. Anal. Calc. for $C_{25}H_{27}N_5O_1 \cdot 2.5\ HCl \cdot 1.05\ H_2O$: C, 57.34; H, 6.08; N, 13.38. Found: C, 57.34; H, 6.03; N, 13.11.

EXAMPLE 19

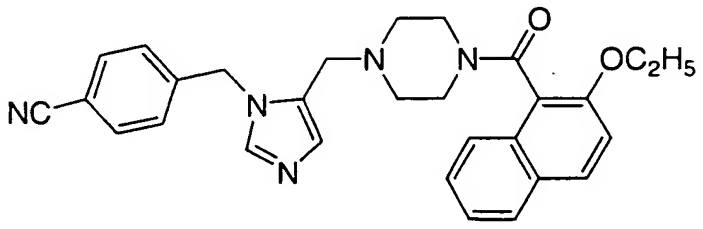


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(8-quinolinoyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 44.8 mg, 0.115 mmol), 8-quinoline carboxylic acid (19.8 mg, 0.115 mmol), EDC hydrochloride (24.2 mg, 0.126 mmol), HOBT (17.0 mg, 0.126 mmol), and N,N-diisopropylethylamine (99.8 μL, 0.573 mmol) were stirred in dry, degassed DMF (1 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 437. Anal. Calc. for $C_{26}H_{24}N_6O_1 \cdot 4.5\ HCl \cdot 0.10\ H_2O$: C, 51.84; H, 4.80; N, 13.95. Found: C, 51.83; H, 4.81; N, 13.73.

EXAMPLE 20



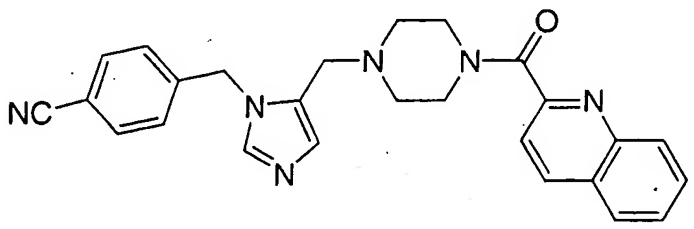
Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-ethoxy-1-naphthoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 40.7 mg, 0.104 mmol), 2-ethoxy-1-naphthoic acid (22.5 mg, 0.104 mmol), EDC hydrochloride (22.0 mg, 0.115 mmol), HOBT (15.5 mg, 0.115 mmol), and N,N-diisopropylethylamine (90.8 μL, 0.521 mmol) were stirred in dry,

degassed DMF (1 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 480. Anal. Calc. for $C_{29}H_{29}N_5O_2 \cdot 2.5\ HCl \cdot 1.0\ H_2O$: C, 59.16; H, 5.74; N, 11.90. Found: C, 59.14; H, 5.39; N, 11.80.

EXAMPLE 21

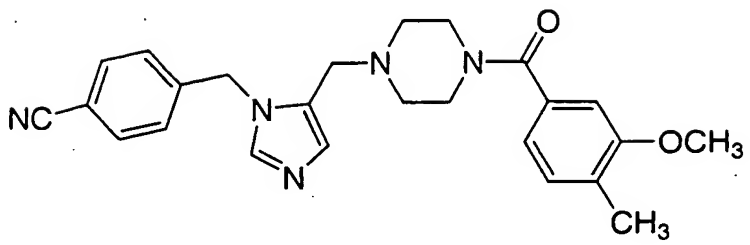


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-quinolinoyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 21.5 mg, 0.0550 mmol), 2-quinoline carboxylic acid (9.52 mg, 0.0550 mmol), EDC hydrochloride (11.6 mg, 0.0605 mmol), HOBT (8.17 mg, 0.0605 mmol), and N,N-diisopropylethylamine (47.9 μL, 0.275 mmol) were stirred in dry, degassed DMF (0.5 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 437. Anal. Calc. for $C_{26}H_{24}N_6O_1 \cdot 3.9\ HCl \cdot 1.1\ Et_2O$: C, 55.30; H, 5.94; N, 12.73. Found: C, 55.30; H, 6.05; N, 12.72.

EXAMPLE 22

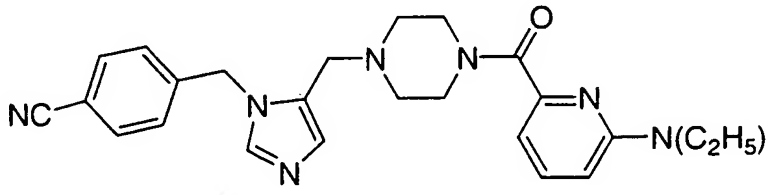


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methoxy-4-methylbenzoyl)piperazine bishydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 23.3 mg, 0.0597 mmol), 3-methoxy-4-methylbenzoic acid (9.92 mg, 0.0597 mmol), EDC hydrochloride (12.6 mg, 0.0657 mmol), HOBT (8.88 mg, 0.0657 mmol), and N,N-diisopropylethylamine (52.0 μL, 0.299 mmol) were stirred in dry, degassed DMF (0.5 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 430. Anal. Calc. for $C_{25}H_{27}N_5O_2 \cdot 2.7\ HCl \cdot 1.9\ H_2O$: C, 53.67; H, 6.01; N, 12.52. Found: C, 53.64; H, 6.00; N, 12.37.

EXAMPLE 23



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine trihydrochloride

Step 1: Preparation of 6-diethylaminopyridine-2-ethyl ester

A solution of 6-chlorpyridine-2-carboxylic acid (2.5 g, 15.9 mmol) and diethylamine (25ml , 64.8 mmol) in ethanol (25ml) was placed and shaked well in a steal bomb reaction vessel at 200°C for 4 hrs. The solvent was removed *in vacuo* and the residue was treated with triethyl amine (3 x 10ml) and concentrated *in vacuo* to yield the title compound which was sufficiently pure for use in the next step.

Step 2: Preparation of 6-diethylaminopyridine-2-carboxylic acid

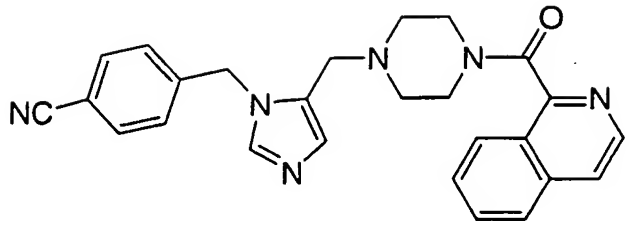
The ester from step 1 (2g, 9.0 mmol) and NaOH (1M, 50ml) were stirred in MeOH (50ml) at reflux for 3hrs. The reaction was concentrated *in vacuo*. The residue was dissolved in methylene chloride (15ml) and HCl (1M in ether, 5ml) was added. The solvent was removed *in vacuo*. The crude product was purified by a C18 preparative HPLC column with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min and the title compound was isolated.

Step 3: Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (Example 2, Step 2, 150 mg, 0.38 mmol), the acid from Step 2 (200 mg, 0.769 mmol), EDC hydrochloride (150 mg, 0.0.769 mmol), HOBT (0.350 mg, 0.38 mmol), and triethyl amine (264 µL, 1.9 mmol) were stirred in dry DMF (4 ml) at 20°C under Argon. Another portion of the piperazine (Example 2, Step 2, 80mg, 0.205 mmol) was added after 5 min and the reaction was stirred at 20°C for 1 hr. The reaction was concentrated *in vacuo* and then worked up with ethyl acetate and H_2O. The crude product was purified by a C18 preparative HPLC column with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 458. Anal. Calc. for $C_{26}H_{31}N_7O \cdot 3.0\ HCl \cdot 0.55\ H_2O$: C, 54.13; H, 6.13; N, 17.00. Found: C, 54.12; H, 6.62; N, 15.05.

EXAMPLE 24

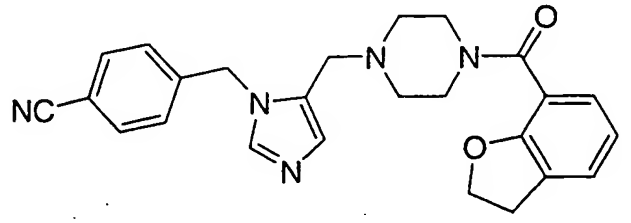


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-isoquinolinoyl)piperazine trihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (21.5 mg, 0.0550 mmol), prepared as described in Example 2, Step 2, 1-isoquinolinecarboxylic acid (9.52 mg, 0.0550 mmol), EDC hydrochloride (11.6 mg, 0.0605 mmol), HOBT (8.17 mg, 0.0605 mmol), and N,N-diisopropylethylamine (47.9 mL, 0.275 mmol) were stirred in dry, degassed DMF (500 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 437. Anal. Calc. for $C_{26}H_{24}N_6O_1 \cdot 4.5\ HCl \cdot 0.45\ Et_2O$: C, 52.82; H, 5.25; N, 13.30. Found: C, 52.78; H, 5.42; N, 13.30.

EXAMPLE 25



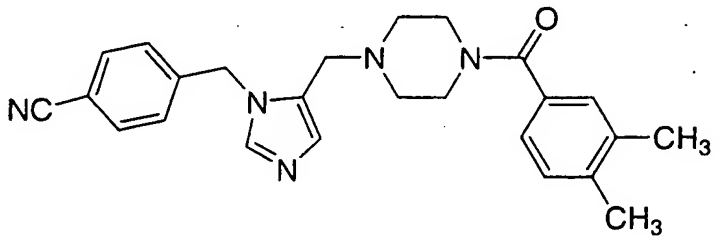
Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2,3-dihydrobenzofuran-7-oyl)piperazine dihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (23.4 mg, 0.0600 mmol), prepared as described in Example 2, Step 2, 2,3-dihydrobenzofuran-7-carboxylic acid (9.84 mg, 0.0600 mmol), EDC hydrochloride

(12.6 mg, 0.0659 mmol), HOBT (8.91 mg, 0.0659 mmol), and N,N-diisopropylethylamine (52.2 mL, 0.300 mmol) were stirred in dry, degassed DMF (500 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 428. Anal. Calc. for $C_{25}H_{25}N_5O_2 \cdot 3.2\ HCl \cdot 0.40\ Et_2O$: C, 55.50; H, 5.65; N, 12.17. Found: C, 55.49; H, 5.80; N, 12.34.

EXAMPLE 26

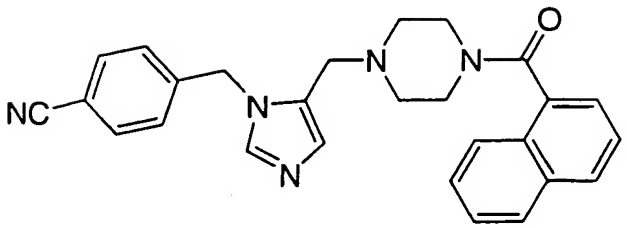


Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,4-dimethylbenzoyl) piperazine dihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (20.1 mg, 0.0514 mmol), prepared as described in Example 2, Step 2, 3,4-dimethylbenzoic acid (7.72 mg, 0.0514 mmol), EDC hydrochloride (10.8 mg, 0.0565 mmol), HOBT (7.64 mg, 0.0565 mmol), and N,N-diisopropylethylamine (44.8 mL, 0.257 mmol) were stirred in dry, degassed DMF (500 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 414. Anal. Calc. for $C_{25}H_{27}N_5O_1 \cdot 2.5\ HCl \cdot 1.35\ H_2O$: C, 56.76; H, 6.14; N, 13.24. Found: C, 56.81; H, 5.87; N, 13.04.

EXAMPLE 27



Preparation of 4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-naphthoyl)piperazine dihydrochloride

1-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]piperazine trihydrochloride (26.9 mg, 0.0688 mmol), prepared as described in Example 2, Step 2, 1-naphthoic acid (11.9 mg, 0.0688 mmol), EDC hydrochloride (14.5 mg, 0.0758 mmol), HOBT (10.2 mg, 0.0758 mmol), and N,N-diisopropylethylamine (60.0 mL, 0.344 mmol) were stirred in dry, degassed DMF (500 mL) at 20°C under nitrogen. The reaction was stirred overnight and then injected onto a C18 preparative HPLC column and purified with a mixed gradient of 5%-95% acetonitrile/0.1% TFA; 95%-5%/0.1% aqueous TFA over 15 min. The title compound was isolated after conversion to the hydrochloride salt.

ES ms (m+1) 436. Anal. Calc. for $C_{27}H_{25}N_5O_1 \cdot 2.8$ HCl: C, 60.11; H, 5.20; N, 12.98. Found: C, 60.06; H, 5.15; N, 13.06.

EXAMPLE 28

In vitro inhibition of ras farnesyl transferase

Transferase Assays. Isoprenyl-protein transferase activity assays are carried out at 30°C unless noted otherwise. A typical reaction contains (in a final volume of 50 μL): [^{3}H]farnesyl diphosphate, Ras protein , 50 mM HEPES, pH 7.5, 5 mM $MgCl_2$, 5 mM dithiothreitol, 10 μM $ZnCl_2$, 0.1% polyethyleneglycol (PEG) (15,000-20,000 mw) and isoprenyl-protein transferase. The FPTase employed in the assay is prepared by recombinant expression as described in Omer, C.A., Kral, A.M., Diehl, R.E., Prendergast, G.C., Powers, S., Allen, C.M., Gibbs, J.B. and Kohl, N.E. (1993) Biochemistry 32:5167-5176. After thermally pre-equilibrating the assay mixture in the absence of enzyme, reactions are initiated by the addition of isoprenyl-

protein transferase and stopped at timed intervals (typically 15 min) by the addition of 1 M HCl in ethanol (1 mL). The quenched reactions are allowed to stand for 15 m (to complete the precipitation process). After adding 2 mL of 100% ethanol, the reactions are vacuum-filtered through Whatman GF/C filters. Filters are washed four times with 2 mL aliquots of 100% ethanol, mixed with scintillation fluid (10 mL) and then counted in a Beckman LS3801 scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 20-fold into the enzyme assay mixture. Substrate concentrations for inhibitor IC_{50} determinations are as follows: FTase, 650 nM Ras-CVLS (SEQ.ID.NO.: 1), 100 nM farnesyl diphosphate.

The compounds of the instant invention described in the above Examples 3-27 were tested for inhibitory activity against human FPTase by the assay described above and were found to have an IC_{50} of ≤ 5 μM.

EXAMPLE 29

Modified In vitro GGTase inhibition assay

The modified geranylgeranyl-protein transferase inhibition assay is carried out at room temperature. A typical reaction contains (in a final volume of 50 μL): [^{3}H]geranylgeranyl diphosphate, biotinylated Ras peptide, 50 mM HEPES, pH 7.5, a modulating anion (for example 10 mM glycerophosphate or 5mM ATP), 5 mM $MgCl_2$, 10 μM $ZnCl_2$, 0.1% PEG (15,000-20,000 mw), 2 mM dithiothreitol, and geranylgeranyl-protein transferase type I(GGTase). The GGTase-type I enzyme employed in the assay is prepared as described in U.S. Pat. No. 5,470,832, incorporated by reference. The Ras peptide is derived from the K4B-Ras protein and has the following sequence: biotinyl-GKKKKKKSKTKCVIM (single amino acid code) (SEQ.ID.NO.: 2). Reactions are initiated by the addition of GGTase and stopped at timed intervals (typically 15 min) by the addition of 200 μL of a 3 mg/mL suspension of streptavidin SPA beads (Scintillation Proximity Assay beads, Amersham) in 0.2 M sodium phosphate, pH 4, containing 50 mM EDTA, and 0.5% BSA. The quenched reactions are allowed to stand for 2 hours before analysis on a Packard TopCount scintillation counter.

For inhibition studies, assays are run as described above, except inhibitors are prepared as concentrated solutions in 100% dimethyl sulfoxide and then diluted 25-fold into the enzyme assay mixture. IC_{50} values are determined with Ras peptide near K_M concentrations. Enzyme and substrate concentrations for inhibitor IC_{50} determinations are as follows: 75 pM GGTase-I, 1.6 μM Ras peptide, 100 nM geranylgeranyl diphosphate.

The compounds of the instant invention described in the above Examples 3-27 were tested for inhibitory activity against human GGTase-type I by the assay described above and were found to have an IC_{50} of ≤ 500 nM.

EXAMPLE 30

Cell-based in vitro ras farnesylation assay

The cell line used in this assay is a v-ras line derived from either Rat1 or NIH3T3 cells, which expressed viral Ha-ras p21. The assay is performed essentially as described in DeClue, J.E. et al., Cancer Research 51:712-717, (1991). Cells in 10 cm dishes at 50-75% confluency are treated with the test compound (final concentration of solvent, methanol or dimethyl sulfoxide, is 0.1%). After 4 hours at 37°C, the cells are labeled in 3 ml methionine-free DMEM supple-mented with 10% regular DMEM, 2% fetal bovine serum and 400 μCi[^{35}S]methionine (1000 Ci/mmol). After an additional 20 hours, the cells are lysed in 1 ml lysis buffer (1% NP40/20 mM HEPES, pH 7.5/5 mM $MgCl_2$/1mM DTT/10 mg/ml aprotinen/2 mg/ml leupeptin/2 mg/ml antipain/0.5 mM PMSF) and the lysates cleared by centrifugation at 100,000 x g for 45 min. Aliquots of lysates containing equal numbers of acid-precipitable counts are bought to 1 ml with IP buffer (lysis buffer lacking DTT) and immunoprecipitated with the ras-specific monoclonal antibody Y13-259 (Furth, M.E. et al., J. Virol. 43:294-304, (1982)). Following a 2 hour antibody incubation at 4°C, 200 μl of a 25% suspension of protein A-Sepharose coated with rabbit anti rat IgG is added for 45 min. The immunoprecipitates are washed four times with IP buffer (20 nM HEPES, pH 7.5/1 mM EDTA/1% Triton X-100.0.5% eoxycholate/0.1%/SDS/0.1 M NaCl) boiled in SDS-PAGE sample buffer and loaded on 13% acrylamide gels. When the dye front reached the bottom, the gel is fixed, soaked in Enlightening, dried and autoradiographed. The intensities of the bands corresponding to farnesylated and nonfarnesylated ras proteins are compared to determine the percent inhibition of farnesyl transfer to protein.

EXAMPLE 31

Cell-based in vitro growth inhibition assay

To determine the biological consequences of FPTase inhibition, the effect of the compounds of the instant invention on the anchorage-independent growth of Rat1 cells transformed with either a v-ras, v-raf, or v-mos oncogene is tested. Cells transformed by v-Raf and v-Mos maybe included in the analysis to evaluate the specificity of instant compounds for Ras-induced cell transformation.

Rat 1 cells transformed with either v-ras, v-raf, or v-mos are seeded at a density of 1 x 10^4 cells per plate (35 mm in diameter) in a 0.3% top agarose layer in medium A (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum) over a bottom agarose layer (0.6%). Both layers contain 0.1% methanol or an appropriate concentration of the instant compound (dissolved in methanol at 1000 times the final concentration used in the assay). The cells are fed twice weekly with 0.5 ml of medium A containing 0.1% methanol or the concentration of the instant compound. Photomicrographs are taken 16 days after the cultures are seeded and comparisons are made.

EXAMPLE 32

Construction of SEAP reporter plasmid pDSE100

The SEAP reporter plasmid, pDSE100 was constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from the plasmid pSEAP2-Basic (Clontech, Palo Alto, CA). The plasmid pCMV-RE-AKI was constructed by Deborah Jones (Merck) and contains 5 sequential copies of the 'dyad symmetry response element' cloned upstream of a 'CAT-TATA' sequence derived from the cytomegalovirus immediate early promoter. The plasmid also contains a bovine growth hormone poly-A sequence.

The plasmid, pDSE100 was constructed as follows. A restriction fragment encoding the SEAP coding sequence was cut out of the plasmid pSEAP2-Basic using the restriction enzymes EcoR1 and HpaI. The ends of the linear DNA fragments were filled in with the Klenow fragment of E. coli DNA Polymerase I. The

'blunt ended' DNA containing the SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1694 base pair fragment. The vector plasmid pCMV-RE-AKI was linearized with the restriction enzyme Bgl-II and the ends filled in with Klenow DNA Polymerase I. The SEAP DNA fragment was blunt end ligated into the pCMV-RE-AKI vector and the ligation products were transformed into DH5-alpha E. coli cells (Gibco-BRL). Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid contains the SEAP coding sequence downstream of the DSE and CAT-TATA promoter elements and upstream of the BGH poly-A sequence.

Alternative Construction of SEAP reporter plasmid, pDSE101

The SEAP repotrer plasmid, pDSE101 is also constructed by ligating a restriction fragment containing the SEAP coding sequence into the plasmid pCMV-RE-AKI. The SEAP gene is derived from plasmid pGEM7zf(-)/SEAP.

The plasmid pDSE101 was constructed as follows: A restriction fragment containing part of the SEAP gene coding sequence was cut out of the plasmid pGEM7zf(-)/SEAP using the restriction enzymes Apa I and KpnI. The ends of the linear DNA fragments were chewed back with the Klenow fragment of E. coli DNA Polymerase I. The "blunt ended" DNA containing the truncated SEAP gene was isolated by electrophoresing the digest in an agarose gel and cutting out the 1910 base pair fragment. This 1910 base pair fragment was ligated into the plasmid pCMV-RE-AKI which had been cut with Bgl-II and filled in with E. coli Klenow fragment DNA polymerase. Recombinant plasmids were screened for insert orientation and sequenced through the ligated junctions. The plasmid pCMV-RE-AKI is derived from plasmid pCMVIE-AKI-DHFR (Whang , Y., Silberklang, M., Morgan, A., Munshi, S., Lenny, A.B., Ellis, R.W., and Kieff, E. (1987) J. Virol., 61, 1796-1807) by removing an EcoRI fragment containing the DHFR and Neomycin markers. Five copies of the fos promoter serum response element were inserted as described previously (Jones, R.E., Defeo-Jones, D., McAvoy, E.M., Vuocolo, G.A., Wegrzyn, R.J., Haskell, K.M. and Oliff, A. (1991) Oncogene, 6, 745-751) to create plasmid pCMV-RE-AKI.

The plasmid pGEM7zf(-)/SEAP was constructed as follows. The SEAP gene was PCRed, in two segments from a human placenta cDNA library (Clontech) using the following oligos.

Sense strand N-terminal SEAP : 5' GAGAGGGAATTCGGGCCCTTCCTGCAT GCTGCTGCTGCTGCTGCTGCTGGGC 3' (SEQ.ID.NO.:3)

Antisense strand N-terminal SEAP: 5' GAGAGAGCTCGAGGTTAACCCGGGT GCGCGGCGTCGGTGGT 3' (SEQ.ID.NO.:4)

Sense strand C-terminal SEAP: 5' GAGAGAGTCTAGAGTTAACCCGTGGTCC CCGCGTTGCTTCCT 3' (SEQ.ID.NO.:5)

Antisense strand C-terminal SEAP: 5' GAAGAGGAAGCTTGGTACCGCCACTG GGCTGTAGGTGGTGGCT 3' (SEQ.ID.NO.:6)

The N-terminal oligos (SEQ.ID.NO.: 4 and SEQ.ID.NO.: 5) were used to generate a 1560 bp N-terminal PCR product that contained EcoRI and HpaI restriction sites at the ends. The Antisense N-terminal oligo (SEQ.ID.NO.: 4) introduces an internal translation STOP codon within the SEAP gene along with the HpaI site. The C-terminal oligos (SEQ.ID.NO.: 5 and SEQ.ID.NO.: 6) were used to amplify a 412 bp C-terminal PCR product containing HpaI and HindIII restriction sites. The sense strand C-terminal oligo (SEQ.ID.NO.: 5) introduces the internal STOP codon as well as the HpaI site. Next, the N-terminal amplicon was digested with EcoRI and HpaI while the C-terminal amplicon was digested with HpaI and HindIII. The two fragments comprising each end of the SEAP gene were isolated by electrophoresing the digest in an agarose gel and isolating the 1560 and 412 base pair fragments. These two fragments were then co-ligated into the vector pGEM7zf(-) (Promega) which had been restriction digested with EcoRI and HindIII and isolated on an agarose gel. The resulting clone, pGEM7zf(-)/SEAP contains the coding sequence for the SEAP gene from amino acids.

Construction of a constitutively expressing SEAP plasmid pCMV-SEAP

An expression plasmid constitutively expressing the SEAP protein was created by placing the sequence encoding a truncated SEAP gene downstream of the cytomegalovirus (CMV) IE-1 promoter. The expression plasmid also includes the CMV intron A region 5' to the SEAP gene as well as the 3' untranslated region of the bovine growth hormone gene 3' to the SEAP gene.

The plasmid pCMVIE-AKI-DHFR (Whang et al, 1987) containing the CMV immediate early promoter was cut with EcoRI generating two fragments. The vector fragment was isolated by agarose electrophoresis and religated. The resulting plasmid is named pCMV-AKI. Next, the cytomegalovirus intron A nucleotide sequence was inserted downstream of the CMV IE1 promter in pCMV-AKI. The intron A sequence was isolated from a genomic clone bank and subcloned into pBR322 to generate plasmid p16T-286. The intron A sequence was mutated at nucleotide 1856 (nucleotide numbering as in Chapman, B.S., Thayer, R.M., Vincent, K.A. and Haigwood, N.L., Nuc.Acids Res. 19, 3979-3986) to remove a SacI restriction site using site directed mutagenesis. The mutated intron A sequence was PCRed from the plasmid p16T-287 using the following oligos.

Sense strand: 5' GGCAGAGCTCGTTTAGTGAACCGTCAG 3' (SEQ.ID.NO.: 7)

Antisense strand: 5' GAGAGATCTCAAGGACGGTGACTGCAG 3' (SEQ.ID.NO.: 8)

These two oligos generate a 991 base pair fragment with a SacI site incorporated by the sense oligo and a Bgl-II fragment incorporated by the antisense oligo. The PCR fragment is trimmed with SacI and Bgl-II and isolated on an agarose gel. The vector pCMV-AKI is cut with SacI and Bgl-II and the larger vector fragment isolated by agarose gel electrophoresis. The two gel isolated fragments are ligated at their respective SacI and Bgl-II sites to create plasmid pCMV-AKI-InA.

The DNA sequence encoding the truncated SEAP gene is inserted into the pCMV-AKI-InA plasmid at the Bgl-II site of the vector. The SEAP gene is cut out of plasmid pGEM7zf(-)/SEAP (described above) using EcoRI and HindIII. The fragment is filled in with Klenow DNA polymerase and the 1970 base pair fragment

isolated from the vector fragment by agarose gel electrophoresis. The pCMV-AKI-InA vector is prepared by digesting with Bgl-II and filling in the ends with Klenow DNA polymerase. The final construct is generated by blunt end ligating the SEAP fragment into the pCMV-AKI-InA vector. Transformants were screened for the proper insert and then mapped for restriction fragment orientation. Properly oriented recombinant constructs were sequenced across the cloning junctions to verify the correct sequence. The resulting plasmid, named pCMV-SEAP, contains a modified SEAP sequence downstream of the cytomegalovirus immediately early promoter IE-1 and intron A sequence and upstream of the bovine growth hormone poly-A sequence. The plasmid expresses SEAP in a constitutive manner when transfected into mammalian cells.

Cloning of a Myristylated viral-H-ras expression plasmid

A DNA fragment containing viral-H-ras can be PCRed from plasmid "H-1" (Ellis R. et al. J. Virol. 36, 408, 1980) or "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) using the following oligos.

Sense strand:

5'TCTCCTCGAGGCCACCATGGGGAGTAGCAAGAGCAAGCCTAAGGACCC CAGCCAGCGCCGGATGACAGAATACAAGCTTGTGGTGG 3'. (SEQ.ID.NO.: 9)

Antisense:

5'CACATCTAGATCAGGACAGCACAGACTTGCAGC 3'.
(SEQ.ID.NO.: 10)

A sequence encoding the first 15 aminoacids of the v-src gene, containing a myristylation site, is incorporated into the sense strand oligo. The sense strand oligo also optimizes the 'Kozak' translation initiation sequence immediately 5' to the ATG start site.To prevent prenylation at the viral-ras C-terminus, cysteine 186 would be mutated to a serine by substituting a G residue for a C residue in the C-

terminal antisense oligo. The PCR primer oligos introduce an XhoI site at the 5' end and a XbaI site at the 3'end. The XhoI-XbaI fragment can be ligated into the mammalian expression plasmid pCI (Promega) cut with XhoI and XbaI. This results in a plasmid in which the recombinant myr-viral-H-ras gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of a viral-H-ras-CVLL expression plasmid

A viral-H-ras clone with a C-terminal sequence encoding the amino acids CVLL can be cloned from the plasmid "H-1" (Ellis R. et al. J. Virol. 36, 408, 1980) or "HB-11 (deposited in the ATCC under Budapest Treaty on August 27, 1997, and designated ATCC 209,218) by PCR using the following oligos.

Sense strand:

5'TCTCCTCGAGGCCACCATGACAGAATACAAGCTTGTGGTGG-3' (SEQ.ID.NO.: 11)

Antisense strand:

5'CACTCTAGACTGGTGTCAGAGCAGCACACACTTGCAGC-3' (SEQ.ID.NO.: 12)

The sense strand oligo optimizes the 'Kozak' sequence and adds an XhoI site. The antisense strand mutates serine 189 to leucine and adds an XbaI site. The PCR fragment can be trimmed with XhoI and XbaI and ligated into the XhoI-XbaI cut vector pCI (Promega). This results in a plasmid in which the mutated viral-H-ras-CVLL gene is constitutively transcribed from the CMV promoter of the pCI vector.

Cloning of c-H-ras-Leu61 expression plasmid

The human c-H-ras gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:

5'-GAGAGAATTCGCCACCATGACGGAATATAAGCTGGTGG-3'
(SEQ.ID.NO.: 13)

Antisense strand:

5'-GAGAGTCGACGCGTCAGGAGAGCACACACTTGC-3' (SEQ.ID.NO.: 14)

The primers will amplify a c-H-ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-H-ras fragment can be ligated ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glutamine-61 to a leucine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-CCGCCGGCCTGGAGGAGTACAG-3' (SEQ.ID.NO.: 15)

After selection and sequencing for the correct nucleotide substitution, the mutated c-H-ras-Leu61 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid will constitutively transcribe c-H-ras-Leu61 from the CMV promoter of the pCI vector.

Cloning of a c-N-ras-Val-12 expression plasmid

The human c-N-ras gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:

5'-GAGAGAATTCGCCACCATGACTGAGTACAAACTGGTGG-3'
(SEQ.ID.NO.: 16)

Antisense strand:

5'-GAGAGTCGACTTGTTACATCACCACACATGGC-3' (SEQ.ID.NO.: 17)

The primers will amplify a c-N-ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, an EcoRI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with EcoRI and Sal I, the c-N-ras fragment can be ligated into an EcoRI -Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of glycine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTTGGAGCAGTTGGTGTTGGG-3' (SEQ.ID.NO.: 18)

After selection and sequencing for the correct nucleotide substitution, the mutated c-N-ras-Val-12 can be excised from the pAlter-1 vector, using EcoRI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with EcoRI and Sal I. The new recombinant plasmid will constitutively transcribe c-N-ras-Val-12 from the CMV promoter of the pCI vector.

Cloning of a c-K-ras-Val-12 expression plasmid

The human c-K-ras gene can be PCRed from a human cerebral cortex cDNA library (Clontech) using the following oligonucleotide primers.

Sense strand:

5'-GAGAGGTACCGCCACCATGACTGAATATAAACTTGTGG-3'
(SEQ.ID.NO.: 19)

Antisense strand:

5'-CTCTGTCGACGTATTTACATAATTACACACTTTGTC-3' (SEQ.ID.NO.: 20)

The primers will amplify a c-K-ras encoding DNA fragment with the primers contributing an optimized 'Kozak' translation start sequence, a KpnI site at the N-terminus and a Sal I stite at the C-terminal end. After trimming the ends of the PCR product with Kpn I and Sal I, the c-K-ras fragment can be ligated into a KpnI - Sal I cut mutagenesis vector pAlter-1 (Promega). Mutation of cysteine-12 to a valine can be accomplished using the manufacturer's protocols and the following oligonucleotide:

5'-GTAGTTGGAGCTGTTGGCGTAGGC-3' (SEQ.ID.NO.: 21)

After selection and sequencing for the correct nucleotide substitution, the mutated c-K-ras-Val-12 can be excised from the pAlter-1 vector, using KpnI and Sal I, and be directly ligated into the vector pCI (Promega) which has been digested with KpnI and Sal I. The new recombinant plasmid will constitutively transcribe c-K-ras-Val-12 from the CMV promoter of the pCI vector.

SEAP assay

Human C33A cells (human epitheial carcenoma - ATTC collection) are seeded in 10cm tissue culture plates in DMEM + 10% fetal calf serum + 1X Pen/Strep + 1X glutamine + 1X NEAA. Cells are grown at 37°C in a 5% CO_2 atmosphere until they reach 50 -80% of confluency.

The transient transfection is performed by the $CaPO_4$ method (Sambrook et al., 1989). Thus, expression plasmids for H-ras, N-ras, K-ras, Myr-ras or H-ras-CVLL are co-precipitated with the DSE-SEAP reporter construct. For 10cm plates 600µl of $CaCl_2$ -DNA solution is added dropwise while vortexing to 600µl of 2X HBS buffer to give 1.2ml of precipitate solution (see recipes below). This is allowed to sit at room temperature for 20 to 30 minutes. While the precipitate is forming, the media on the C33A cells is replaced with DMEM (minus phenol red; Gibco cat. # 31053-028)+ 0.5% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and nonessential aminoacids). The $CaPO_4$-DNA precipitate is added dropwise to the cells and the plate rocked gently to distribute. DNA uptake is allowed to proceed for 5-6 hrs at 37°C under a 5% CO_2 atmosphere.

Following the DNA incubation period, the cells are washed with PBS and trypsinized with 1ml of 0.05% trypsin. The 1 ml of trypsinized cells is diluted

into 10ml of phenol red free DMEM + 0.2% charcoal stripped calf serum + 1X (Pen/Strep, Glutamine and NEAA). Transfected cells are plated in a 96 well microtiter plate (100µl/well) to which drug, diluted in media, has already been added in a volume of 100µl. The final volume per well is 200µl with each drug concentration repeated in triplicate over a range of half-log steps.

Incubation of cells and drugs is for 36 hrs at 37°Cunder CO_2. At the end of the incubation period, cells are examined microscopically for evidence of cell distress. Next, 100µl of media containing the secreted alkaline phosphatase is removed from each well and transferred to a microtube array for heat treatment at 65°C for 1 hr to inactivate endogenous alkaline phosphatases (but not the heat stable secreted phosphatase).

The heat treated media is assayed for alkaline phosphatase by a luminescence assay using the luminescence reagent CSPD®(Tropix, Bedford, Mass.). A volume of 50 µl media is combined with 200 µl of CSPD cocktail and incubated for 60 minutes at room temperature. Luminesence is monitored using an ML2200 microplate luminometer (Dynatech). Luminescence reflects the level of activation of the fos reporter construct stimulated by the transiently expressed protein.

DNA-$CaPO_4$ precipitate for 10cm. plate of cells

Ras expression plasmid (1µg/µl)	10µl
DSE-SEAP Plasmid (1µg/µl)	2µl
Sheared Calf Thymus DNA (1µg/µl)	8µl
2M $CaCl_2$	74µl
dH_2O	506µl

2X HBS Buffer

280mM NaCl
10mM KCl
1.5mM Na_2HPO_4 $2H_2O$
12mM dextrose
50mM HEPES
Final pH = 7.05

Luminesence Buffer (26ml)

Assay Buffer	20ml
Emerald Reagent™ (Tropix)	2.5ml
100mM homoarginine	2.5ml
CSPD Reagent® (Tropix)	1.0ml

Assay Buffer

Add 0.05M Na_2CO_3 to 0.05M $NaHCO_3$ to obtain pH 9.5.

Make 1mM in $MgCl_2$

EXAMPLE 33

The processing assays employed are modifications of that described by DeClue et al [Cancer Research 51, 712-717, 1991].

K4B-Ras processing inhibition assay

PSN-1 (human pancreatic carcinoma) or viral-K4B-ras-transformed Rat1 cells are used for analysis of protein processing. Subconfluent cells in 100 mm dishes are fed with 3.5 ml of media (methionine-free RPMI supplemented with 2% fetal bovine serum or cysteine-free/methionine-free DMEM supplemented with 0.035 ml of 200 mM glutamine (Gibco), 2% fetal bovine serum, respectively) containing the desired concentration of test compound, lovastatin or solvent alone. Cells treated with lovastatin (5-10 μM), a compound that blocks Ras processing in cells by inhibiting a rate-limiting step in the isoprenoid biosynthetic pathway, serve as a positive control. Test compounds are prepared as 1000x concentrated solutions in DMSO to yield a final solvent concentration of 0.1%. Following incubation at 37°C for two hours 204 μCi/ml [^{35}S]Pro-Mix (Amersham, cell labeling grade) is added.

After introducing the label amino acid mixture, the cells are incubated at 37°C for an additional period of time (typically 6 to 24 hours). The media is then removed and the cells are washed once with cold PBS. The cells are scraped into 1 ml of cold PBS, collected by centrifugation (10,000 x g for 10 sec at room temperature), and lysed by vortexing in 1 ml of lysis buffer (1% Nonidet P-40, 20 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% deoxycholate, 0.1% SDS, 1 mM DTT, 10 μg/ml AEBSF, 10 μg/ml aprotinin, 2 μg/ml leupeptin and 2 μg/ml

antipain). The lysate is then centrifuged at 15,000 x g for 10 min at 4°C and the supernatant saved.

For immunoprecipitation of Ki4B-Ras, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 8 μg of the pan Ras monoclonal antibody, Y13-259, added. The protein/antibody mixture is incubated on ice at 4°C for 24 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer 0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Kirsten-ras specific monoclonal antibody, c-K-ras Ab-1 (Calbiochem). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) coated with rabbit antiserum to rat IgG (Cappel) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Ras is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Ras visualized by fluorography.

hDJ processing inhibition assay

PSN-1 cells are seeded in 24-well assay plates. For each compound to be tested, the cells are treated with a minimum of seven concentrations in half-log steps. The final solvent (DMSO) concentration is 0.1%. A vehicle-only control is included on each assay plate. The cells are treated for 24 hours at 37•C / 5% CO_2.

The growth media is then aspirated and the samples are washed with PBS. The cells are lysed with SDS-PAGE sample buffer containing 5% 2-

mercaptoethanol and heated to 95•C for 5 minutes. After cooling on ice for 10 minutes, a mixture of nucleases is added to reduce viscosity of the samples.

The plates are incubated on ice for another 10 minutes. The samples are loaded onto pre-cast 8% acrylamide gels and electrophoresed at 15 mA/gel for 3-4 hours. The samples are then transferred from the gels to PVDF membranes by Western blotting.

The membranes are blocked for at least 1 hour in buffer containing 2% nonfat dry milk. The membranes are then treated with a monoclonal antibody to hDJ-2 (Neomarkers Cat. # MS-225), washed, and treated with an alkaline phosphatase-conjugated secondary antibody. The membranes are then treated with a fluorescent detection reagent and scanned on a phosphorimager.

For each sample, the percent of total signal corresponding to the unprenylated species of hDJ (the slower-migrating species) is calculated by densitometry. Dose-response curves and EC_{50} values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 34

Rap1 processing inhibition assay

Protocol A:

Cells are labeled, incubated and lysed as described in Example 33. For immunoprecipitation of Rap1, samples of lysate supernatant containing equal amounts of protein are utilized. Protein concentration is determined by the bradford method utilizing bovine serum albumin as a standard. The appropriate volume of lysate is brought to 1 ml with lysis buffer lacking DTT and 2 μg of the Rap1 antibody, Rap1/Krev1 (121) (Santa Cruz Biotech) is added. The protein/antibody mixture is incubated on ice at 4°C for 1 hour. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in 100 μl elution buffer (10 mM Tris pH 7.4, 1% SDS). The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation (15,000 x g for 30 sec. at room temperature).

The supernatant is added to 1 ml of Dilution Buffer (0.1% Triton X-100, 5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.4) with 2 μg Rap1 antibody,

Rap1/Krev1 (121) (Santa Cruz Biotech). The second protein/antibody mixture is incubated on ice at 4°C for 1-2 hours. The immune complex is collected on pansorbin (Calbiochem) by tumbling at 4°C for 45 minutes. The pellet is washed 3 times with 1 ml of lysis buffer lacking DTT and protease inhibitors and resuspended in Laemmli sample buffer. The Rap1 is eluted from the beads by heating at 95°C for 5 minutes, after which the beads are pelleted by brief centrifugation. The supernatant is subjected to SDS-PAGE on a 12% acrylamide gel (bis-acrylamide:acrylamide, 1:100), and the Rap1 visualized by fluorography.

Protocol B:

PSN-1 cells are passaged every 3-4 days in 10cm plates, splitting near-confluent plates 1:20 and 1:40. The day before the assay is set up, 5x 10^6 cells are plated on 15cm plates to ensure the same stage of confluency in each assay. The media for these cells is RPM1 1640 (Gibco), with 15% fetal bovine serum and 1x Pen/Strep antibiotic mix.

The day of the assay, cells are collected from the 15cm plates by trypsinization and diluted to 400,000 cells/ml in media. 0.5ml of these diluted cells are added to each well of 24-well plates, for a final cell number of 200,000 per well. The cells are then grown at 37°C overnight.

The compounds to be assayed are diluted in DMSO in 1/2-log dilutions. The range of final concentrations to be assayed is generally 0.1-100μM. Four concentrations per compound is typical. The compounds are diluted so that each concentration is 1000x of the final concentration (i.e., for a 10μM data point, a 10mM stock of the compound is needed).

2μL of each 1000x compound stock is diluted into 1ml media to produce a 2X stock of compound. A vehicle control solution (2μL DMSO to 1ml media), is utilized. 0.5 ml of the 2X stocks of compound are added to the cells.

After 24 hours, the media is aspirated from the assay plates. Each well is rinsed with 1ml PBS, and the PBS is aspirated. 180μL SDS-PAGE sample buffer (Novex) containing 5% 2-mercapto-ethanol is added to each well. The plates are heated to 100•C for 5 minutes using a heat block containing an adapter for assay plates. The plates are placed on ice. After 10 minutes, 20μL of an RNAse/DNase mix is added per well. This mix is 1mg/ml DNaseI (Worthington Enzymes), 0.25mg/ml Rnase A (Worthington Enzymes), 0.5M Tris-HCl pH8.0 and 50mM

$MgCl_2$. The plate is left on ice for 10 minutes. Samples are then either loaded on the gel, or stored at -70•C until use.

Each assay plate (usually 3 compounds, each in 4-point titrations, plus controls) requires one 15-well 14% Novex gel. 25μl of each sample is loaded onto the gel. The gel is run at 15mA for about 3.5 hours. It is important to run the gel far enough so that there will be adequate separation between 21kd (Rap1) and 29kd (Rab6).

The gels are then transferred to Novex pre-cut PVDF membranes for 1.5 hours at 30V (constant voltage). Immediately after transferring, the membranes are blocked overnight in 20ml Western blocking buffer (2% nonfat dry milk in Western wash buffer (PBS + 0.1% Tween-20). If blocked over the weekend, 0.02% sodium azide is added. The membranes are blocked at 4•C with slow rocking.

The blocking solution is discarded and 20ml fresh blocking solution containing the anti Rap1a antibody (Santa Cruz Biochemical SC1482) at 1:1000 (diluted in Western blocking buffer) and the anti Rab6 antibody (Santa Cruz Biochemical SC310) at 1:5000 (diluted in Western blocking buffer) are added. The membranes are incubated at room temperature for 1 hour with mild rocking. The blocking solution is then discarded and the membrane is washed 3 times with Western wash buffer for 15 minutes per wash. 20ml blocking solution containing 1:1000 (diluted in Western blocking buffer) each of two alkaline phosphatase conjugated antibodies (Alkaline phosphatase conjugated Anti-goat IgG and Alkaline phosphatase conjugated anti-rabbit IgG [Santa Cruz Biochemical]) is then added. The membrane is incubated for one hour and washed 3x as above.

About 2ml per gel of the Amersham ECF detection reagent is placed on an overhead transparency (ECF) and the PVDF membranes are placed face-down onto the detection reagent. This is incubated for one minute, then the membrane is placed onto a fresh transparency sheet.

The developed transparency sheet is scanned on a phosphorimager and the Rap1a Minimum Inhibitory Concentration is determined from the lowest concentration of compound that produces a detectable Rap1a Western signal. The Rap1a antibody used recognizes only unprenylated/unprocessed Rap1a, so that the precence of a detectable Rap1a Western signal is indicative of inhibition of Rap1a prenylation.

Protocol C

This protocol allows the determination of an EC_{50} for inhibition of processing of Rap1a. The assay is run as described in Protocol B with the following modifications. 20 μl of sample is run on pre-cast 10-20% gradient acrylamide mini gels (Novex Inc.) at 15 mA/gel for 2.5-3 hours. Prenylated and unprenylated forms of Rap1a are detected by blotting with a polyclonal antibody (Rap1/Krev-1 b#121;Santa Cruz Research Products #sc-65), followed by an alkaline phosphatase-conjugated anti-rabbit IgG antibody. The percentage of unprenylated Rap1a relative to the total amount of Rap1a is determined by peak integration using Imagequant7 software (Molecular Dynamics). Unprenylated Rap1a is distinguished from prenylated protein by virtue of the greater apparent molecular weight of the prenylated protein. Dose-response curves and EC_{50} values are generated using 4-parameter curve fits in SigmaPlot software.

EXAMPLE 35

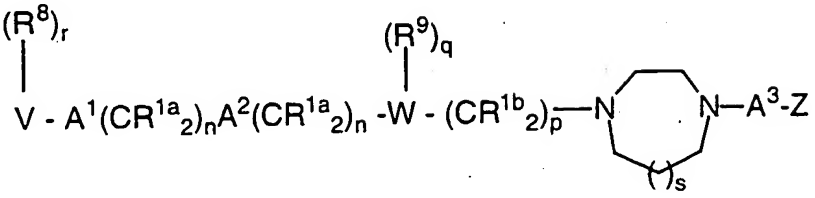
In vivo tumor growth inhibition assay (nude mouse)

In vivo efficacy as an inhibitor of the growth of cancer cells may be confirmed by several protocols well known in the art. Examples of such in vivo efficacy studies are described by N. E. Kohl et al. (Nature Medicine, 1:792-797 (1995)) and N. E. Kohl et al. (Proc. Nat. Acad. Sci. U.S.A., 91:9141-9145 (1994)).

Rodent fibroblasts transformed with oncogenically mutated human Ha-ras or Ki-ras (10^6 cells/animal in 1 ml of DMEM salts) are injected subcutaneously into the left flank of 8-12 week old female nude mice (Harlan) on day 0. The mice in each oncogene group are randomly assigned to a vehicle, compound or combination treatment group. Animals are dosed subcutaneously starting on day 1 and daily for the duration of the experiment. Alternatively, the farnesyl-protein transferase inhibitor may be administered by a continuous infusion pump. Compound, compound combination or vehicle is delivered in a total volume of 0.1 ml. Tumors are excised and weighed when all of the vehicle-treated animals exhibited lesions of 0.5 - 1.0 cm in diameter, typically 11-15 days after the cells were injected. The average weight of the tumors in each treatment group for each cell line is calculated.

WHAT IS CLAIMED IS:

1. A compound which is:



A

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, NO_2, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-,

c) unsubstituted or substituted C_1-C_6 alkyl wherein the substituent on the substituted C_1-C_6 alkyl is selected from unsubstituted or substituted aryl, heterocyclic, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, and $R^{11}OC(O)$-NR^{10}-;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, unsubstituted or substituted heterocycle, unsubstituted or substituted C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, NO_2, $R^{10}C(O)$-, N_3, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, cyanophenyl, heterocycle, C_3-C_{10} cycloalkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NH$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{10}OC(O)NH$-;

R^9 is selected from:

a) hydrogen,

b) alkenyl, alkynyl, perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, NO_2, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, and

c) C_1-C_6 alkyl unsubstituted or substituted by perfluoroalkyl, F, Cl, Br, $R^{10}O$-, $R^{11}S(O)_m$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2NC(O)$-, $R^{10}{}_2N$-$C(NR^{10})$-, CN, $R^{10}C(O)$-, N_3, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, -$NR^{10}C(O)$-, O, -$N(R^{10})$-, -$S(O)_2N(R^{10})$-, -$N(R^{10})S(O)_2$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

e) C_2-C_{20} alkenyl, provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

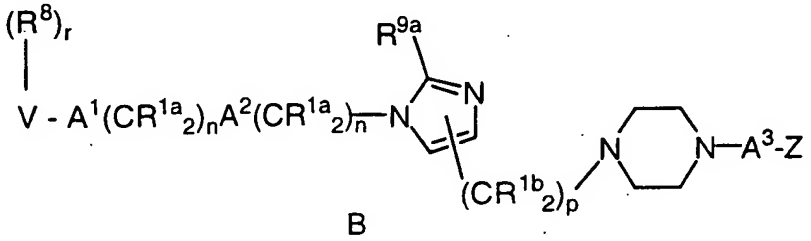
W is a heterocycle;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is 0, 1, 2, 3 or 4;
q is 1 or 2;
r is 0 to 5, provided that r is 0 when V is hydrogen; and
s is 0 or 1,

or the pharmaceutically acceptable salts thereof.

2. The compound according to Claim 1 of the formula B:



B

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,
b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN,

NO_2, $(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-C(NR^{10})-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, O, -$N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

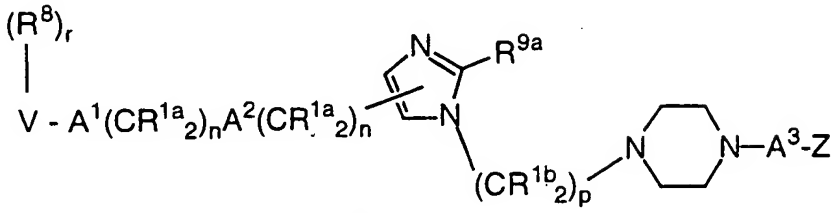
e) C_2-C_{20} alkenyl, and provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is	0, 1 or 2;
n is	0, 1, 2, 3 or 4;
p is	0, 1, 2, 3 or 4; and
r is	0 to 5, provided that r is 0 when V is hydrogen;

or the pharmaceutically acceptable salts thereof.

3. The compound according to Claim 1 of the formula C:



C

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, -$N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or -$N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 and A^2 are independently selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -C(O)NR^{10}-, O, -$N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) hydrogen,

b) heterocycle selected from pyrrolidinyl, imidazolyl, pyridinyl, thiazolyl, pyridonyl, 2-oxopiperidinyl, indolyl, quinolinyl, isoquinolinyl, and thienyl,

c) aryl,

d) C_1-C_{20} alkyl wherein from 0 to 4 carbon atoms are replaced with a heteroatom selected from O, S, and N, and

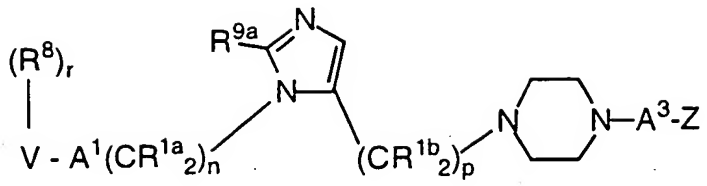
e) C_2-C_{20} alkenyl, and provided that V is not hydrogen if A^1 is $S(O)_m$ and V is not hydrogen if A^1 is a bond, n is 0 and A^2 is $S(O)_m$;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is	0, 1 or 2;
n is	0, 1, 2, 3 or 4;
p is	2, 3 or 4; and
r is	0 to 5, provided that r is 0 when V is hydrogen;

or the pharmaceutically acceptable salts thereof.

4. The compound according to Claim 2 of the formula D:



D

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 is selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, $-C(O)NR^{10}$-, O, $-N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) heterocycle selected from pyridinyl and quinolinyl, and

b) aryl;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;

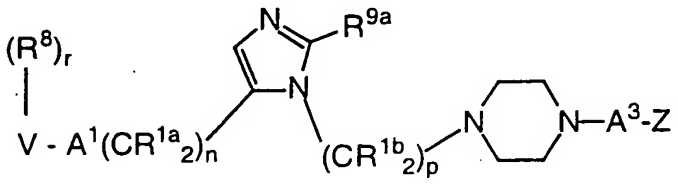
n is 0, 1, 2, 3 or 4;

p is 0, 1, 2, 3 or 4; and

r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

5. The compound according to Claim 3 of the formula E:



E

wherein:

R^{1a} and R^{1b} are independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, -$C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, -$N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^1 is selected from: a bond, -CH=CH-, -C≡C-, -C(O)-, -$C(O)NR^{10}$-, O, -$N(R^{10})$-, or $S(O)_m$;

A^3 is selected from: -C(O)- or $S(O)_m$;

V is selected from:

a) heterocycle selected from pyridinyl and quinolinyl, and

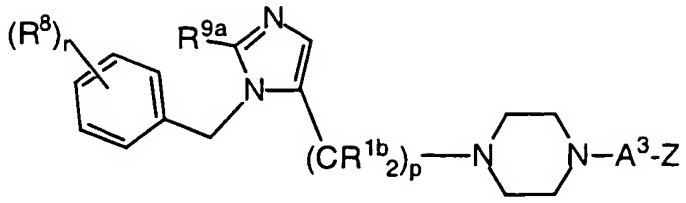
b) aryl;

Z is unsubstituted or substituted aryl or unsubstituted or substituted heteroaryl;

m is 0, 1 or 2;
n is 0, 1, 2, 3 or 4;
p is 2, 3 or 4; and
r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

6. The compound according to Claim 4 of the formula F:



F

wherein:

R^{1b} is independently selected from:

a) hydrogen,

b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,

c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,

b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and

c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^3 is -C(O)-;

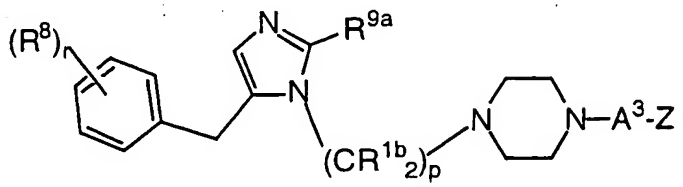
Z is unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted 2,3-

dihydrobenzofuran, unsubstituted or substituted quinoline or unsubstituted or substituted isoquinoline;

p is 1, 2 or 3; and
r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

7. The compound according to Claim 5 of the formula G:



G

wherein:

R^{1b} is independently selected from:

a) hydrogen,
b) aryl, heterocycle, cycloalkyl, $R^{10}O$-, $-N(R^{10})_2$ or C_2-C_6 alkenyl,
c) C_1-C_6 alkyl unsubstituted or substituted by aryl, heterocycle, cycloalkyl, alkenyl, $R^{10}O$-, or $-N(R^{10})_2$;

R^8 is independently selected from:

a) hydrogen,
b) unsubstituted or substituted aryl, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_6 perfluoroalkyl, F, Cl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, CN, NO_2, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-, $-C(O)OR^{10}$ and
c) C_1-C_6 alkyl substituted by C_1-C_6 perfluoroalkyl, $R^{10}O$-, $R^{10}C(O)NR^{10}$-, $(R^{10})_2N$-$C(NR^{10})$-, $R^{10}C(O)$-, $-N(R^{10})_2$, or $R^{11}OC(O)NR^{10}$-;

R^{9a} is hydrogen, C_1-C_6 alkyl or chloro;

R^{10} is independently selected from hydrogen, C_1-C_6 alkyl, benzyl and aryl;

R^{11} is independently selected from C_1-C_6 alkyl and aryl;

A^3 is -C(O)-;

Z is unsubstituted or substituted phenyl, unsubstituted or substituted napthyl, unsubstituted or substituted pyridyl, unsubstituted or substituted 2,3-dihydrobenzofuran, unsubstituted or substituted quinoline or unsubstituted or substituted isoquinoline;

p is 2 or 3; and
r is 0 to 5,

or the pharmaceutically acceptable salts thereof.

8. A compound which is selected from:

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-methoxyquinolin-4-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-diethylamino-3-ethoxypyrid-5-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-ethylamino-4-isoquinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-bromo-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(pent-1-ynyl)-1-naphthoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[5-(prop-1-ynyl)-1-naphthoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-propyl-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-bromo-3-methylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl -4-(prop-1-ynyl)benzoyl]piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methyl-4-pentylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-cyclopropyleth-ynyl-5-methoxybenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-methoxy-2-pent-1-ynylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethynylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(5-chloro-2-cyclohexylethylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(4-indoloyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,5-dimethylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(8-quinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-ethoxy-1-naphthoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2-quinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3-methoxy-4-methylbenzoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-isoquinolinoyl)piperazine

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(2,3-dihydrobenzofuran-7-oyl)piperazine

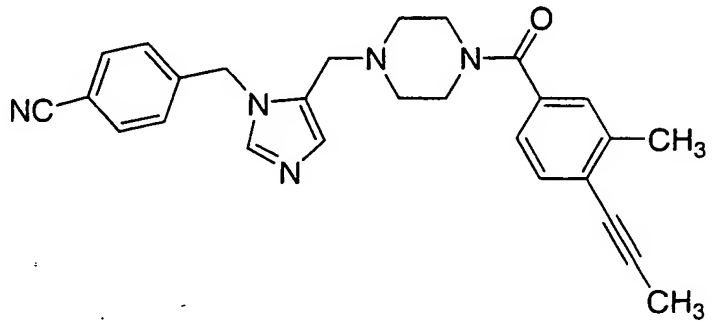
4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(3,4-dimethylbenzoyl) piperazine and

4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-naphthoyl)piperazine

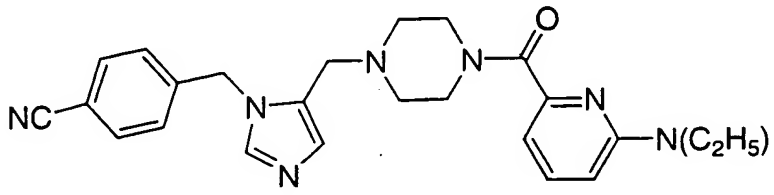
or a pharmaceutically acceptable salt or optical isomer thereof.

9. The compound according to Claim 8 which is

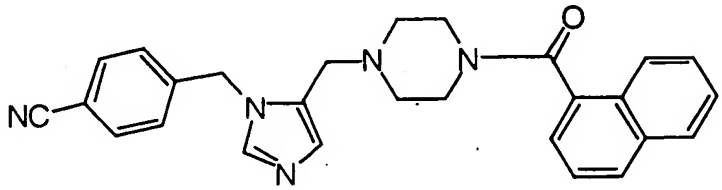
4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-[3-methyl -4-(prop-1-ynyl)benzoyl]piperazine



4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(6-diethylamino-pyrid-2-oyl)piperazine



4-[1-(4-Cyanobenzyl)imidazol-5-ylmethyl]-1-(1-naphthoyl)piperazine



or a pharmaceutically acceptable salt or optical isomer thereof.

10. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 1.

11. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 4.

12. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 5.

13. A pharmaceutical composition comprising a pharmaceutical carrier, and dispersed therein, a therapeutically effective amount of a compound of Claim 8.

14. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

15. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 11.

16. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 12.

17. A method for inhibiting farnesyl-protein transferase and geranylgeranyl-protein transferase type I which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 13.

18. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

19. A method according to Claim 18 wherein the cancer is characterized by a mutated K4B-Ras protein.

20. A method for treating cancer which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 13.

21. A method according to Claim 20 wherein the cancer is characterized by a mutated K4B-Ras protein.

22. A method for treating neurofibromin benign proliferative disorder which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

23. A method for treating blindness related to retinal vascularization which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

24. A method for treating infections from hepatitis delta and related viruses which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

25. A method for preventing restenosis which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

26. A method for treating polycystic kidney disease which comprises administering to a mammal in need thereof a therapeutically effective amount of a composition of Claim 10.

27. A pharmaceutical composition made by combining the compound of Claim 1 and a pharmaceutically acceptable carrier.

28. A process for making a pharmaceutical composition comprising combining a compound of Claim 1 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05354

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/496, 31/551; A 61 P 9/10, 13/12, 27/02, 31/14, 35/00; C 12 N 9/99; C07D 403/04, 403/06, 403/12, 403/14

US CL : 514/218, 253.09; 540/575; 544/360, 364, 370

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/218, 253.09; 540/575; 544/360, 364, 370

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN Substructure Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BE 891580 A1 (KYORIN PHARMACEUTICAL CO.) 16 May 1982 (16.04.1982) see claims 1-4 on page 9.	1, 10, 27, AND 28
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A		2-9, 11-26
Y	US 5,856,326 A (ANTHONY, N.J. ET AL) 05 January 1999 (05.01.1999), see examples 4, 5, 18, 24, 25, 26, 27, and 29-37 in columns 59-75.	1-28
A	OLSON, R.E. ET AL Balanced angiotensin II receptor antagonists II. 4-aminomethyl and acylaminomethylimidazoles, Bioorg. Med. Chem. Lett., 1994, Vol. 4, No. 18, pages 2229-2234.	1-28

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
20 April 2000 (20.04.2000)

Date of mailing of the international search report
27 JUN 2000

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/05354

Box I Observations where certain claims were found unsearchable (Continuati n f Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/05354

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 2-9, 11-13, 15-17, 20, and 21, parts of 1, 10, 14, 18, 19, and 22-28, drawn to piperazines, molecules of formula A with s = zero.

Group II, claim(s) parts of 1, 10, 14, 18, 19, and 22-28, drawn to homopiperazines, molecules of formula A with s = one.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical feature, which is mandatory for both groups, is the heterocyclic ring containing the two nitrogen atoms. The ring is six membered in Group I and seven membered in Group II.